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13. ABSTRACT *(Maximum 200 Words)*

The primary accomplishments of the previous funding period were: 1) cloning and expression of the mouse isoform of Scn10a gene product (SNS; TTX-resistant sodium channel); 2) functional heterologous expression and initial biophysical characterization of the mouse Scn10a gene product in sympathetic neurons; 3) cloning and sequencing of 4 Kbp of upstream genomic DNA; and 4) identification of BAC (bacterial artificial chromosome) clones harboring the mouse Scn10a gene. The nucleotide sequence of the mouse Scn10a cDNA obtained in these studies deviates significantly (170 discrepancies in 5874 bases) from a previously published cDNA sequence derived from genomic DNA (GenBank accession number Y09108) and thus represents new information. Heterologous expression of the Scn10a cDNA in sympathetic neurons revealed a significant depolarizing shift in the biophysical properties of the expressed channel when compared with natively expressed channels. These findings indicate that an accessory protein may influence the behavior of the channel. A candidate promoter region for the Scn10a channel has been cloned and identified in BAC clones. Taken together, these results provide the experimental foundation for our future studies aimed at identifying new potential therapeutic targets that modify the function and expression of sodium channels involved in pain transmission.

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Introduction

The Scn10a gene product encodes a tetrodotoxin-resistant sodium channel (SNS/PN3) expressed exclusively in a subset of primary sensory neurons (e.g., dorsal root and nodose ganglia) believed to be involved in pain transmission (Akopian et al., 1996). Thus, it is important to understand mechanisms contributing to both the function of the protein and the exquisite specificity of gene expression. The overall research plan is detailed in the flowchart depicted to the right. During the last funding period, we have made significant progress on both the genomic (left branch) and proteomic (right branch) sections of the research plan.

Figure 1
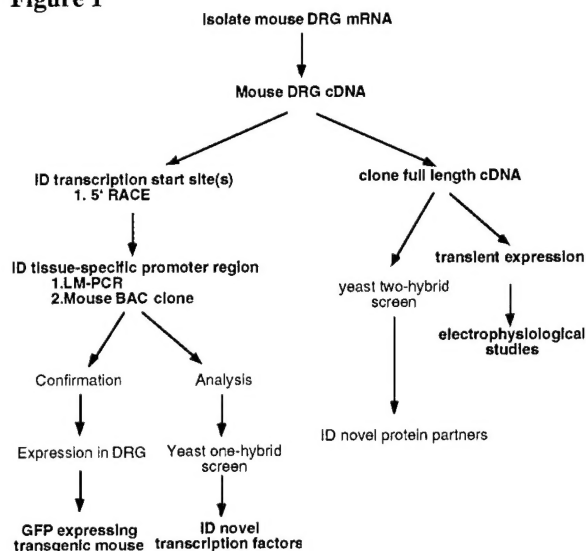
Specifically, we have cloned and sequenced the full length mouse Scn10a cDNA. Moreover, we have demonstrated that the cDNA is functionally expressed in neurons and have initiated biophysical characterization of the expressed channels. With regard to progress toward better understanding the regulation of Scn10a transcription, the putative transcription start site has been identified using 5' rapid amplification of cDNA ends (RACE) and 4 Kbp of upstream genomic region has been sequenced using ligation-mediated PCR (LM-PCR). Finally, bacterial artificial chromosome (BAC) clones containing the full length mouse Scn10a gene have been identified.

Body

A. Cloning of mouse Scn10a cDNA

In order to obtain a murine Scn10a clone, dorsal root ganglion (DRG) neurons were enzymatically dissociated from adult CD1 mice and polyA mRNA isolated by standard techniques. A full length mScn10a clone was amplified from this material by RT-PCR using oligonucleotide primers based on the genomic sequence data provided in Souslova et al. (1997). The open reading frame was subcloned into the mammalian expression vector pCI (Promega) and sequenced with an ABI 377 automated sequencer (Appendix A). The sequence of the Scn10a clone was verified by sequencing the PCR product from several different mRNA preparations.

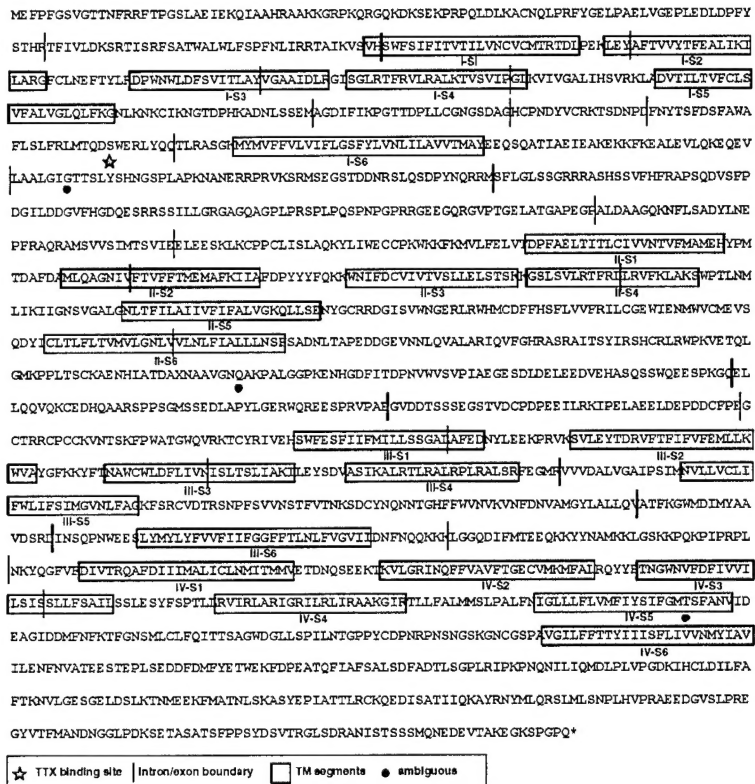The nucleotide sequence of mouse Scn10a cDNA, when compared with sequence (#Y09108) derived from mouse genomic data (Souslova et al., 1997), revealed 170 discrepancies of 5874 total bp (Appendix B). About 50% of the changes do not alter the primary sequence (i.e., are silent mutations). About 1/3 of the "polymorphisms" are A to G changes (genomic to cDNA). An alignment of the primary amino acid sequence of our

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clone compared with #Y09108 is shown in Appendix C. The reason for these discrepancies is unclear.

The derived primary sequence of mouse Scn10a is 93, 82, and 81% identical to the rat (X92184), human (AF117907), and dog (U60590) Scn10A sodium channel, respectively. When conserved substitutions are taken into account (ClustalW analysis), the sequence similarity is 95, 89, and 88%, respectively.

Figure 2, Mouse Scn10a primary amino acid sequence

Putative transmembrane segments are shown in shaded boxes with each color representing one of four homologous domains. The serine residue shown to be involved in TTX insensitivity in the rat ortholog (Sivilotti et al., 1997) is marked with a star. Putative intron/exon boundaries are from Souslova et al., 1997. Ambiguous residues are those not confirmed by sequencing of different clones.

```
MEFPFGSVGTTNFRRFTPGSLAEIEKQIAAHRAAKKGRPKQRGQKDKSEKPRPQLDLKACNQLPRFYGELPAELVGEPLEDLDPFY

STHRTFIVLDKSRTISRFSATWALWLFSPFNLIRRTAIKVSVHSWFSIFITVTILVNCVCMTRTDIPEHLEYAFTVVYTFEALIKI
                                         I-S1              I-S2

LARGFCLNEFTYLRDPWNWLDFSVITLAYVGAAIDLRGISGLRTFRVLRALKTVSVIPGIKVIVGALIHSVRKLADVTILTVFCLS
             I-S3                      I-S4                              I-S5

VFALVGLQLFKGNLKNKCIKNGTDPHKADNLSSEMAGDIFIKPGTTDPLLCGNGSDAGHCPNDYVCRKTSDNPDFNYTSFDSFAWA

FLSLFRLMTQDSWERLYQQTLRASGHMYMVFFVLVIFLGSFYLVNLILAVVTMAYEEQSQATIAEIEAKEKKFKEALEVLQKEQEV
                         I-S6  ☆

LAALGIGTTSLYSHNGSPLAPKNANERRPRVKSRMSEGSTDDNRSLQSDPYNQRRMSFLGLSSGRRRASHSSVFHFRAPSQDVSFP
 ●

DGILDDGVFHGDQESRRSSILLGRGAGQAGPLPRSPLPQSPNPGPRRGEEGQRGVPTGELATGAPEGHALDAAGQKNFLSADYLNE

PFRAQRAMSVVSIMTSVIEELEESKLKCPPCLISLAQKYLIWECCPKWKKFKMVLFELVTDPFAELTITLCIVVNTVFMAMEHYPM
                                                           II-S1

TDAFDAMLQAGNIVFTVFFTMEMAFKIIAFDPYYYFQKWNIFDCVIVTVSLLELSTSRHGSLSVLRTFRILRVFKLAKSWPTLNM
      II-S2                               II-S3              II-S4

LIKIIGNSVGALGNLTFILAIIVFIFALVGKQLLSENYGCRRDGISVWNGERLRWHMCDFFHSFLVVFRILCGEWIENMWVCMEVS
             II-S5

QDYICLTLFLTVMVLGNLVVLNLFIALLLNSFSADNLTAPEDDGEVNNLQVALARIQVFGHRASRAITSYIRSHCRLRWPKVETQL
     II-S6

GMKPPLTSCKAENHIATDAXNAAVGNQAKPALGGPKENHGDFITDPNVWVSVPIAEGESDIDELEEDVEHASQSSWQEESPKGQEL
                   ●

LQQVQKCEDHQAARSPPSGMSSEDLAPYLGERWQREESPRVPAEGVDDTSSSEGSTVDCPDPEEILRKIPELAEEILDEPDDCFPEG

CTRRCPCCKVNTSKFPWATGWQVRKTCYRIVEHSWFESFIIFMILLSSGALAFEDNYLEEKPRVKSVLEYTDRVFTFIFVFEMLLK
                                 III-S1                        III-S2

WVAYGFKKYFTNAWCWLDFLIVNISLTSLIAKILEYSDVASIKALRTLRALRPLRALSRFEGMRVVVDALVGAIPSIMNVLLVCLI
          III-S3                      III-S4

FWLIFSIMGVNLFAGKFSRCVDTRSNPFSVVNSTFVTNKSDCYNQNNTGHFFWVNVKVNFDNVAMGYLALLQVATFKGWMDIMYAA
      III-S5

VDSRDINSQPNWEESLYMYLYFVVFIIFGGFPTLNLFVGVIIDNFNQQKKQLGGQDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPL
               III-S6

NKYQGFVEDIVTRQAFDIIIMALICLNMITMMVETDNQSEEKIKVLGRINQFFVAVFTGECVMKMFALRQYYFTNGWNVFDFIVVI
         IV-S1              IV-S2                 IV-S3

LSISSLLFSAIISSLESYFSPTLFRVIRLARIGRILRLIRAAKGIRTLLPALMMSLPALFNIGLLLFLVMFIYSIFGMTSFANVID
         IV-S4                                      IV-S5  ●

EAGIDDMFNFKTFGNSMLCLFQITTSAGWDGLLSPILNTGPPYCDPNRPNSNGSKGNCGSPAVGILFFTTYIIISFLIVVNMYIAV
                                                         IV-S6

ILENFNVATEESTEPLSEDDFDMFYETWEKFDPEATQFIAFSALSDFADTLSGPLRIPKPNQNILIQMDLPLVPGDKIHCLDILFA

FTKNVLGESGELDSLKTNMEEKFMATNLSKASYEPIATTLRCKQEDISATIIQKAYRNYMLQRSLMLSNPLHVPRAEEDGVSLPRE

GYVTFMANDNGGLPDKSETASATSFPPSYDSVTRGLSDRANISTSSSMQNEDEVTAKEGKSPGPQ*
```

☆ TTX binding site | Intron/exon boundary | □ TM segments | ● ambiguous

B. Expression of Scn10a cDNA clone in rat sympathetic neurons

Rat sympathetic neurons isolated from superior cervical ganglion (SCG) were used as a host for Scn10a expression. Previous studies have shown that natively expressed sodium channels in SCG neurons are completely suppressed by tetrodotoxin (Schofield & Ikeda, 1988). Scn10a (0.1–0.2 µg/µl) and EGFP (Clontech, 0.05 µg/µl) cDNAs were co-injected into the nucleus of SCG neurons using an Eppendorf microinjection system (Ikeda, 1996, 1997). Patch clamp recordings were made following 12-18 hours of incubation at 37 C. Successfully injected cells were identified by EGFP fluorescence using an inverted microscope (Nikon) equipped with an epifluorescence unit.

Voltage-clamp recordings were made at room temperature (23–25 °C) using the whole-cell variant of the patch-clamp technique. Solutions designed to isolate tetrodotoxin resistant (TTX-R) currents were as follows: external (mM): TEA-Cl 120, NaCl 50, HEPES 10, $MnCl_2$ 2, and glucose 10. TTX (1 µM) was added to suppress sensitive

currents. The pH of the solution was adjusted to 7.4 with TEA-OH. The osmolality of the solution was 327 mosm/kg. Internal (mM): CsCl 115, NaCl 10, EGTA 11, $CaCl_2$ 1,HEPES 10, MgATP 4, GTP 0.1, and diTRIS phosphocreatine, 5. The pH of the solution was adjusted to 7.2 with TEA-OH. The osmolality of the solution was 303 mosm/kg. Current-voltage (I-V) and activation curves were derived from currents evoked by a 20 msec test pulse to various potentials from a holding potential of -80 mV. Conductance calculations were made using the chord conductance equation assuming a reversal potential of +40 mV. Inactivation was determined using a 1 sec conditioning pulse followed by a test pulse to 0 mV. Parameters for activation and inactivation curves were determined by fitting the normalized conductance curve to a Boltzmann function using a non-linear regression program. An example of currents and activation and inactivation analysis is shown in Figure 3.

Figure 3. TTX-resistant sodium currents recorded from rat sympathetic neurons expressing Scn10a channels.

Upper right: Family of currents evoked by depolarizing pulses. *Lower left*: Current voltage relationship. *Upper right*: Currents evoked with the inactivation voltage protocol. *Lower right*: Steady state activation and inactivation curves.

Figure 4. TTX-resistant sodium currents recorded from mouse DRG neurons

Upper right: Family of currents evoked by depolarizing pulses. *Lower left*: Current voltage relationship. *Upper right*: Currents evoked with the inactivation voltage protocol. *Lower right*: Steady state activation and inactivation curves.
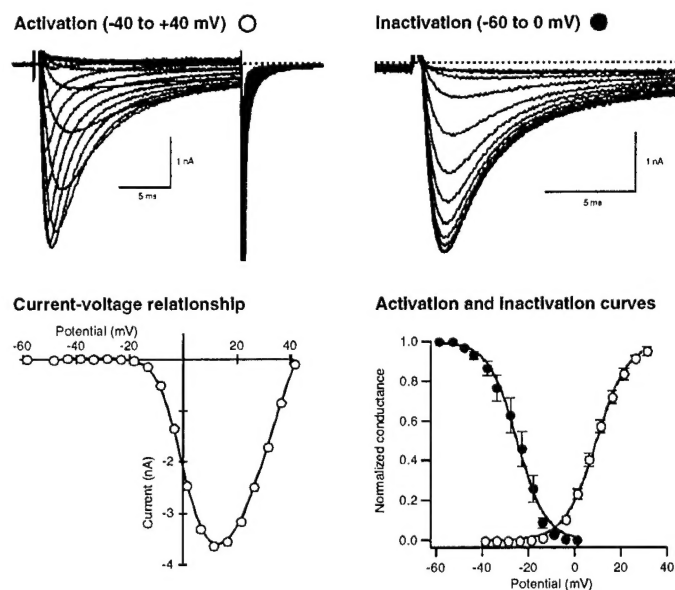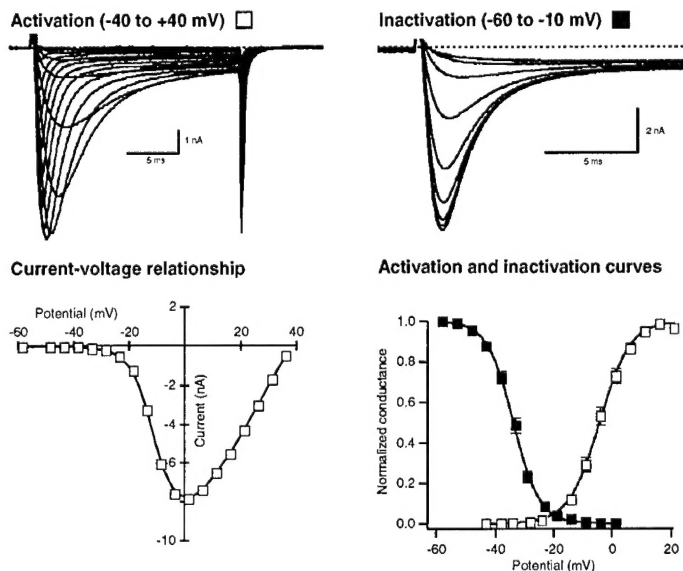

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Tetrodotoxin-resistant sodium currents were also recorded from acutely isolated mouse DRG neurons. The goal of these studies was to compare the heterologously expressed channels with natively expressed channels. An example of such currents is shown above (Figure 4.)

Comparison of heterologously and natively expressed TTX-resistant sodium channels revealed that depolarizing shifts in both the activation and inactivation curves of the cloned channels. Analyses of individual neurons are included in the Appendix (D and E). Mean activation and inactivation curves are depicted in Figure 5.

Figure 5. Mean activation and inactivation curves for natively and heterologously expressed TTX-R Na⁺ channels.

Activation and inactivation curves were fit to the following equation:

$$G(V) = \frac{Gmax}{1 + \exp[(V-Vh)/k]}$$
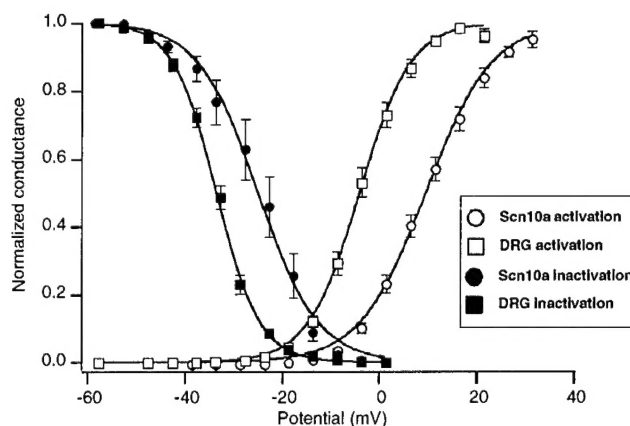
A summary of the mean activation and inactivation parameters derived from the nonlinear regression analyses is show in Table 1.

Table 1.

	Activation		Inactivation	
	Vh_{act} (mV)	k_{act} (mV)	Vh_{inact} (mV)	k_{inact} (mV)
DRG	-4.3 ± 0.9 (9)	5.0 ± 0.2 (9)	-34.0 ± 0.6 (9)	4.3 ± 0.2 (9)
Scn10a	9.4 ± 1.1 (9)**	6.4 ± 0.4 (9)**	-25.2 ± 2.2 (4)	5.6 ± 0.5 (4)

C. Identification of BAC (bacterial artificial chromosome) clones harboring the Scn10a gene

The absolute size of the promoter for the Scn10a gene is unknown. Since other Na⁺ channel genes are regulated by promoters that span greater than 50 kb of genomic DNA, we reasoned that the Scn10a gene promoter may also be very large. To increase the likelihood of isolating the entire promoter, we chose to screen a mouse genomic BAC library prepared by Incyte Genomics. BAC (Bacterial Artificial Chromosome) libraries

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are constructed in specialized plasmid vectors that stably maintain greater than 125 kb of contiguous cloned genomic DNA, and are well-suited for cloning projects where large stretches of genomic DNA need to be examined. Three BAC clones (#26462, #26463, and #26464) were identified in a PCR-based screen using oligonucleotide primers specific to exon 1 of the Scn10a gene.

The clones are currently being analyzed to determine the extent of 5'-flanking DNA (which contains the promoter) carried on each. Preliminary analysis of the three BAC clones by restriction endonuclease analysis indicates that the clones contain contiguous overlapping stretches of mouse genomic DNA containing an undetermined amount of exonic/intronic DNA and 5'-flanking DNA of the Scn10a gene. Pulsed field gel electrophoresis (PFGE) of the clones shows that they are similar, but not identical, in size and are greater than 150 kb (Figure 6). To determine the extent of 5'-untranslated DNA carried on each BAC clone, we are in the process of modifying each clone to contain a unique restriction site, SwaI and/or CeuI, just upstream of the ATG translation codon of the Scn10a gene. Digestion of the modified BAC clones at the unique NotI site in the pBeloBAC II cloning vector and the SwaI and/or CeuI site will release a segment of the BAC DNA corresponding to the 5'-flanking DNA, which will then be sized by PFGE. Incorporation of the SwaI and CeuI sites into each BAC clone is being done using a modified protocol first described in Yang et al. (1997).

2642   2643   26464

Figure 6. Restriction enzyme digestion of BAC DNA. Digestion of the three BAC clones with BamHI or SalI is shown. Lane 1 contains high-molecular weight λ markers
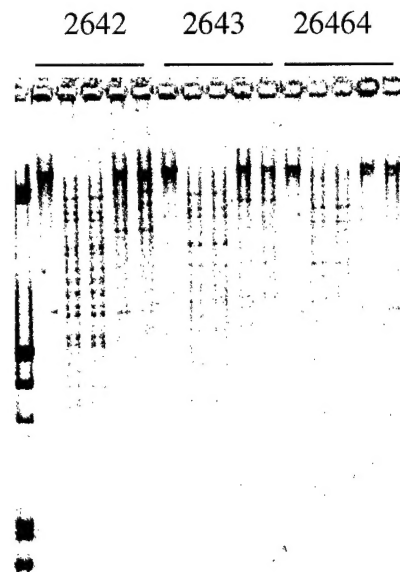
The modification of the BAC clones will also allow us to examine the Scn10a gene promoter in transfected primary neurons as the coding sequence for the enhanced green fluorescent protein (EGFP) will simultaneously be placed immediately downstream of the 5'-flanking region. Expression of the EGFP gene under control of the Scn10a gene promoter is expected to provide a very sensitive read-out of this promoter's activity. In this protocol, a specialized shuttle vector is first constructed *in vitro* that carries a small segment of the 5'-flanking sequence of the Scn10a gene fused to the EGFP gene in the shuttle vector PLD53PA (Figure 7). The shuttle vector is next recombined *in vivo*

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adjacent to the 5'-flanking sequence of the Scn10a gene on the BAC clone, creating a Scn10a promoter-IRES I EGFP fusion in the BAC clone. We have determined the DNA sequence of approximately 900 bp of the 5-flanking region of the Scn10a gene by direct sequence analysis of one of the BACs (Appendix F). We have constructed appropriate pairs of forward and reverse oligonucleotide primers that will be used to amplify by the PCR an approximately 600 bp subfragment (Figure 8). This 600 bp subfragment will be subcloned into the multiple cloning site of the shuttle vector PLD53PA via the NotI and SwaI sites incorporated into the forward and reverse primers. Recombination of the shuttle vector into the BAC DNA will occur at the second IRES I EGFP-PA sequence in PLD53PA. The SacB gene in this vector provides for sucrose-counterselection to select for BAC recombinants that have undergone a recombination event resulting in a final BAC clone containing a stably-integrated Scn10a promoter-EGFP fusion. The resulting BAC clones will retain whatever 5'-flanking DNA that was carried on the unmodified BACs, only fused to IRES I EGFP.

Figure 7. Map of the shuttle vector PLD53PA. The NotI/SwaI sites within PLD53PA for insertion of Scn10a DNA is shown.
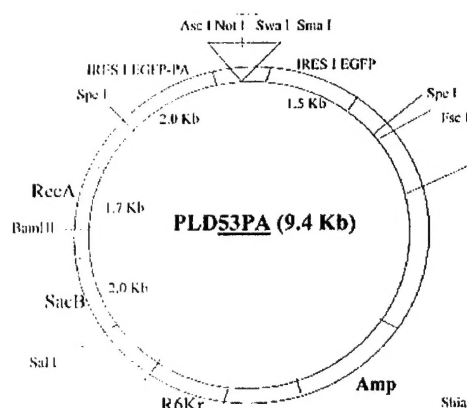
Figure 8. Schematic of the 900 bp 5'-flanking DNA and location of PCR primers. Sets of forward and reverse primers to amplify a 600 bp subregion for insertion into PLD53PA are shown. The specific restriction sites incorporated into each primer is indicated.
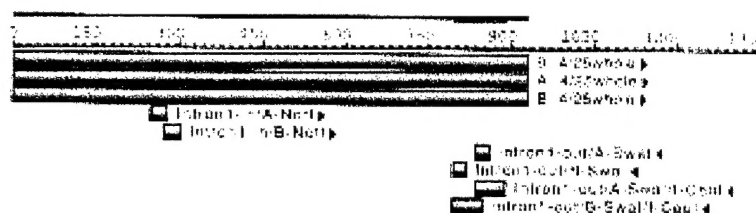
5' RACE Analysis was performed on mRNA isolated using the cellulose based SV total RNA and polyAtract® mRNA isolation systems from Promega. The mRNA was reverse transcribed to first strand cDNA using the Advantage™ RT for PCR kit from Clontech. The cDNA was then and used in the SMART™ RACE cDNA Amplification kit (Clontech) in which the general first strand cDNA synthesis step was followed by a single round of PCR with a universal adaptor primer and a gene specific primer. The gene specific primer was designed to include a portion of the coding sequence for verification

of the authenticity of the product by sequence comparison. Products were visualized and fractionated from a 2.0 % agarose gel stained with ethidium bromide and cloned into the sequencing vector pGEM-T Easy™ (Promega) as per the manufacturer's directions. Sequencing was performed using an ABI 310 and 377 automated sequencer. The reactions produced a number of fragments possibly depending on the number of transcription start sites and the integrity of the cDNA or the mRNA from which it was constructed. The sequences of the resulting RACE products and primers are found in appendix G. A schematic of the two longest clones (B and I) aligned to the 5' end of the Scn10a coding sequence is shown in figure 9.

Figure 9: Alignment of the RACE products B and I to the 5' end of the Scn10a coding sequence
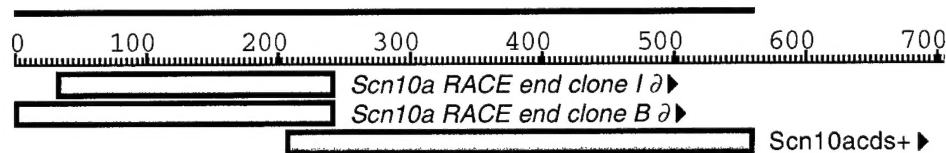
LM-PCR was performed with the use of the Genomewalking™ kit from Clontech. Primers (custom made by Gibco/BRL) were designed to extend into regions of genomic DNA in a 5' direction from the end of the Scn10a gene. The kit supplies genomic DNA cut with blunt end restriction enzymes to which an adaptor has been ligated. The kit supplies primers directed to that adaptor. The adaptor primers are used with gene specific primers in primary and nested PCR reactions. A polymerase mix consisting of *Tth* polymerase and a proofreading polymerase allow
 for the high fidelity amplification of fragments up to 6 kb. PCR fragments from the library or libraries yielding a significant size and purity were further purified from agarose gels and cloned into a sequencing vector (pGEM-T easy™ vector (Promega)) as per the manufacturer's directions. Sequencing was performed using an ABI 310 and 377 automated sequencers. LM-PCR was performed outward from the first protein coding exon about 920 bp into an intron located upstream from this exon and from the upstream ends of the RACE reaction products about 4.0 kb into what we hope is the promoter region of the Scn10a gene. The resulting sequences and primers for the LM-PCR into the 5'UTR intron and the LM-PCR out from the RACE product sequences can be found in appendices H and I respectively. The 4.0 kb fragment was constructed in two steps as shown in figure 10 and is currently being cloned into the EGFP-N1 reporter vector (Clontech) for screening promoter activity. Figure 10 also shows orientation of the 4.0kb fragment with respect to the coding sequence and the 5' RACE products.

Figure 10: Schematic of RACE followed by 2 rounds of LM-PCR (upstream 5' UTR intron has been bypassed: LM-PCR = genomic sequence/RACE and Scn10a cds are mRNA based.)
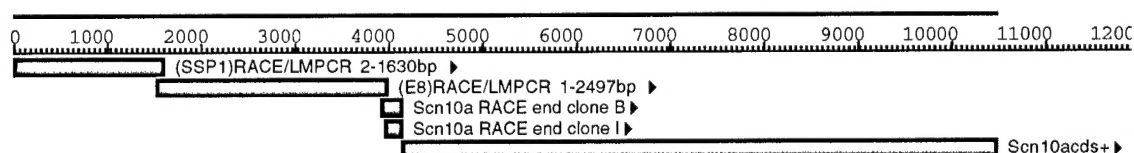
Genomic screening was performed through Incyte Genomics (Palo Alto, CA). The screening was of a mouse genomic library constructed in bacterial artificial chromosomes. The method of screening was PCR based, and primers were designed to the 5' coding sequence (first coding exon) from previously reported sequence information on the mouse genomic sequence (Souslova *et al.*). The screening process identified three unique BAC clones. Many introns have been identified in the mouse genomic Scn10a clone. Introns, generally, are regions of DNA sequence that interrupt the protein-coding portion of a gene. They are spliced out following transcription to yield a continuous coding mRNA. Coding exons on the 5' and 3' ends of the Scn10a gene were used as separate templates for secondary screening of the positives clones we received. The identification of clones yielding positive results for each primer set indicated the presence of the intact coding portion of the gene plus any introns in all three clones. The primers used to PCR screen the mouse genomic library and the sequence of the resulting PCR product are shown in appendix J.

Genomic sequencing was performed on the three clones upon arrival. Clones were supplied as glycerol stocks. The stocks were plated and individual clones were selected with the primers used for the screening protocol to ensure a homogeneous population for future work. We have found that extensive sequencing of the BAC clones is possible with only minor modifications to the protocol supplied with the Perkin Elmer Sequencing kit designed for the ABI 310 and 377 sequence analyzer. The modification involves an increase (10 fold) of DNA template concentration. Initial sequencing with primers directed toward distant regions of the gene such as the 3' and 5' ends were performed as mentioned above to establish the extent of the gene contained in the insert. We have also identified the ends of our RACE reaction and LM-PCR from the RACE reaction products in all three clones indicating that a fair amount of genomic sequence is present beyond the coding exons. Restriction mapping of the BAC clones is also currently underway using a combination of blotting and PCR. Figure 11 contains primer information (A) and alignments of the various regions upon which we have focused in characterizing how intact our BAC clones are with respect to the Scn10a gene. Figure 11 B and C shows sequencing into the 5'UTR intron from both downstream and upstream directions. Figure 11 D shows the direct sequencing of the 3' end of the coding sequence and 11 E shows the direct sequencing of the 5' end of the LM-PCR from RACE products. These figures combined show the presence of the entire coding region of the gene on our BAC clones as well as at least ~4.0 kb above the RACE ends or putative transcriptional start site.

Figure 11: Direct sequencing of BAC clones 26462/26463/26464:

A: Primers:
GTGTAAGTTTCGCAGAGCTGGGGTC-RT B in
TCATGGACAAAGCGTAAGTGC- Scn10a Intron-in1
CCTGCATGCTCTACCAAGTCG- Scn10a Intron-in2
GGTGACAGCCTGACCACTGC- Scn10a Intron-out1
GCTTTGTAAGAAGCTCCATCC- Scn10a Intron-out2
CCTGTGTGTGCTGTAAAAAGGATC - 3' EX-1

B: Sequencing direct in into 5'UTR intron
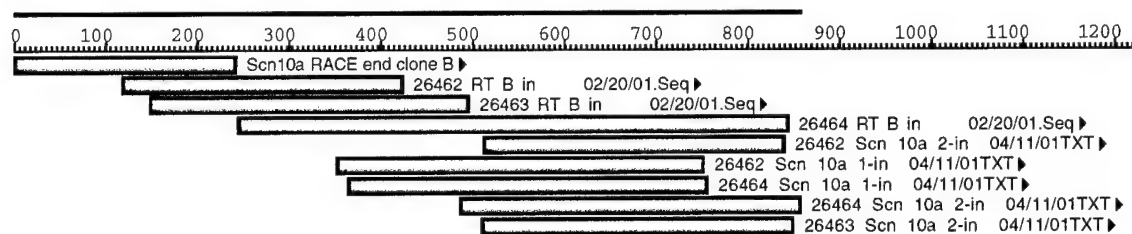
C: Sequencing direct out into 5'UTR intron
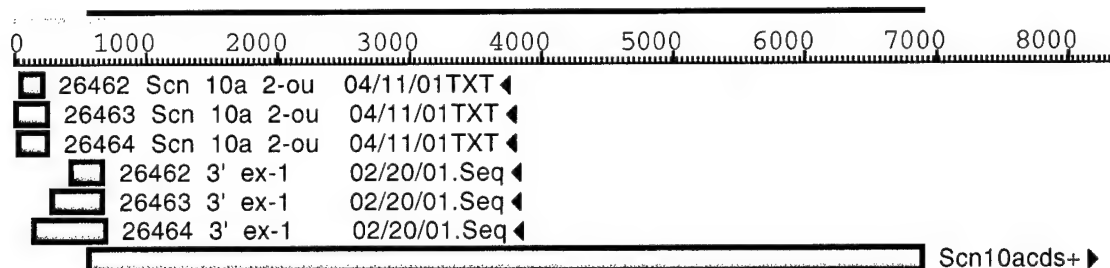
D: 3' end of coding sequence found in all three BAC clones (only BAC clone #26463 shown-sequenced twice txt and txt2)
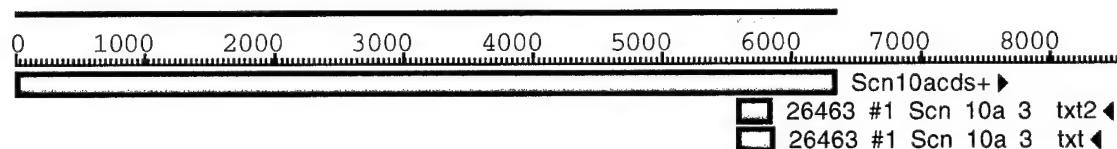
E: 5' end of combined LM-PCR from RACE products (only BAC clone #26463 shown-sequenced twice txt and txt2)
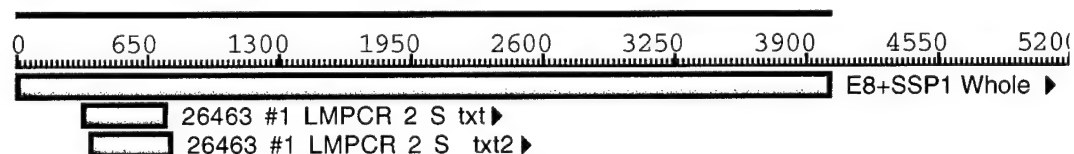
Key Research Accomplishments

- Cloning and sequencing of the mouse Scn10a cDNA
- Functional heterologous expression of mouse Scn10a cDNA in sympathetic neurons
- Biophysical characterization of sodium currents arising from Scn10a expression
- Cloning and sequencing of 4 Kbp of upstream genomic DNA containing putative promoter elements
- Identification of BAC (bacterial artificial chromosome) clones harboring the Scn10a gene

Reportable Outcomes

Abstracts:

1. Ikeda, S.R., King, M.M., Aronstam, R.S. and Puhl, H.L. Cloning and expression of cDNA encoding a tetrodotoxin-resistant (TTX-R) sodium channel (Scn10a) from mouse dorsal root ganglion neurons. *Experimental Biology Meeting,* 2001.

2. Puhl, H.L., King, M.M., Aronstam, R.S. and Ikeda, S.R. Cloning and functional characterization of mouse cDNA encoding a tetrodotoxin-resistant (TTX-R) sodium channel (Scn10a). *Soc. Neurosci. Abstr.,* 2001.

Conclusions

a. A murine ortholog of the TTX-resistant sodium channel Scn10a (SNS/PN3) was cloned from mouse DRG neuron mRNA using the PCR.

b. The predicted protein sequence is highly homologous to the rat, dog, and human Scn10a gene product. Surprisingly, the nucleotide sequence deviates significantly from a published mouse genomic sequence perhaps suggesting a gene duplication.

c. Intranuclear injection of the cloned cDNA into rat sympathetic neurons results in robust TTX-R sodium currents with kinetics characteristic of TTX-R sodium currents recorded from mouse DRG neurons.

d. Steady-state inactivation and activation curves for the heterologously expressed Scn10a sodium current were shifted toward more positive potentials when compared with native DRG TTX-R sodium currents. This suggests that accessory proteins or post-translational modifications may be required to recapitulate the native phenotype.

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Appendix A
Mouse Scn10a cDNA nucleotide sequence

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         10             20             30             40             50             60
ATG GAG TTC CCC TTT GGG TCC GTG GGA ACT ACC AAC TTC AGA CGG TTC ACT CCA GGG TCG
 M   E   F   P   F   G   S   V   G   T   T   N   F   R   R   F   T   P   G   S>

         70             80             90            100            110            120
CTG GCA GAG ATC GAG AAG CAG ATC GCT GCC CAC CGC GCC GCC AAG AAG GGC AGA CCT AAG
 L   A   E   I   E   K   Q   I   A   A   H   R   A   A   K   K   G   R   P   K>

        130            140            150            160            170            180
CAA AGA GGA CAG AAG GAC AAG AGT GAG AAG CCC AGG CCT CAG TTG GAC TTG AAG GCC TGT
 Q   R   G   Q   K   D   K   S   E   K   P   R   P   Q   L   D   L   K   A   C>

        190            200            210            220            230            240
AAC CAG CTG CCC AGG TTC TAT GGC GAG CTC CCA GCA GAG CTG GTC GGG GAG CCC CTG GAG
 N   Q   L   P   R   F   Y   G   E   L   P   A   E   L   V   G   E   P   L   E>

        250            260            270            280            290            300
GAC CTG GAT CCT TTC TAC AGC ACA CAC CGG ACA TTC ATA GTG TTG GAT AAA AGC AGG ACC
 D   L   D   P   F   Y   S   T   H   R   T   F   I   V   L   D   K   S   R   T>

        310            320            330            340            350            360
ATT TCC AGA TTC AGT GCC ACT TGG GCT CTG TGG CTC TTC AGT CCC TTC AAC CTG ATC AGA
 I   S   R   F   S   A   T   W   A   L   W   L   F   S   P   F   N   L   I   R>

        370            380            390            400            410            420
AGA ACA GCC ATC AAA GTG TCC GTC CAC TCC TGG TTC TCC ATA TTT ATC ACT GTC ACT ATT
 R   T   A   I   K   V   S   V   H   S   W   F   S   I   F   I   T   V   T   I>

        430            440            450            460            470            480
TTG GTC AAC TGT GTG TGC ATG ACC CGA ACT GAT CTT CCA GAG AAA CTC GAG TAT GCC TTC
 L   V   N   C   V   C   M   T   R   T   D   L   P   E   K   L   E   Y   A   F>

        490            500            510            520            530            540
ACT GTT GTT TAC ACC TTC GAG GCT CTG ATA AAG ATA CTG GCA AGA GGG TTT TGT CTA AAT
 T   V   V   Y   T   F   E   A   L   I   K   I   L   A   R   G   F   C   L   N>

        550            560            570            580            590            600
GAA TTC ACT TAT CTT CGA GAT CCC TGG AAC TGG CTG GAC TTC AGT GTC ATT ACC CTG GCG
 E   F   T   Y   L   R   D   P   W   N   W   L   D   F   S   V   I   T   L   A>

        610            620            630            640            650            660
TAT GTG GGT GCA GCG ATA GAC CTC CGA GGA ATC TCA GGC CTG CGG ACA TTC CGA GTT CTC
 Y   V   G   A   A   I   D   L   R   G   I   S   G   L   R   T   F   R   V   L>

        670            680            690            700            710            720
AGG GCC CTG AAG ACT GTT TCT GTG ATC CCA GGA CTG AAG GTC ATC GTG GGA GCC CTG ATC
 R   A   L   K   T   V   S   V   I   P   G   L   K   V   I   V   G   A   L   I>

        730            740            750            760            770            780
CAC TCA GTG AGG AAG CTG GCC GAC GTG ACC ATC CTC ACA GTC TTC TGC CTG AGT GTC TTT
 H   S   V   R   K   L   A   D   V   T   I   L   T   V   F   C   L   S   V   F>

        790            800            810            820            830            840
GCC TTG GTG GGC CTG CAG CTC TTC AAG GGG AAC CTC AAG AAT AAA TGC ATT AAG AAC GGC
 A   L   V   G   L   Q   L   F   K   G   N   L   K   N   K   C   I   K   N   G>

        850            860            870            880            890            900
ACA GAT CCG CAC AAG GCT GAC AAT CTC TCA TCT GAA ATG GCA GGA GAC ATC TTC ATC AAG
 T   D   P   H   K   A   D   N   L   S   S   E   M   A   G   D   I   F   I   K>

        910            920            930            940            950            960
CCC GGT ACT ACG GAT CCC TTG TTG TGT GGC AAT GGA TCT GAT GCT GGC CAC TGC CCT AAT
 P   G   T   T   D   P   L   L   C   G   N   G   S   D   A   G   H   C   P   N>

        970            980            990           1000           1010           1020
GAT TAT GTC TGC CGG AAA ACT TCT GAC AAC CCG GAT TTT AAC TAC ACC AGC TTT GAT TCC
 D   Y   V   C   R   K   T   S   D   N   P   D   F   N   Y   T   S   F   D   S>
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       1030          1040          1050          1060          1070          1080
TTC GCG TGG GCG TTC CTC TCA CTG TTC CGT CTC ATG ACG CAG GAC TCC TGG GAA CGG CTG
 F   A   W   A   F   L   S   L   F   R   L   M   T   Q   D   S   W   E   R   L>

              1090          1100          1110          1120          1130          1140
TAC CAG CAG ACA CTC CGG GCT TCC GGG AAA ATG TAC ATG GTC TTT TTT GTG CTG GTC ATC
 Y   Q   Q   T   L   R   A   S   G   K   M   Y   M   V   F   F   V   L   V   I>

              1150          1160          1170          1180          1190          1200
TTC CTT GGA TCA TTC TAC CTG GTC AAT TTG ATC TTG GCT GTG GTC ACC ATG GCA TAT GAG
 F   L   G   S   F   Y   L   V   N   L   I   L   A   V   V   T   M   A   Y   E>

              1210          1220          1230          1240          1250          1260
GAA CAG AGC CAG GCA ACA ATT GCA GAA ATC GAA GCC AAG GAG AAG AAG TTC AAG GAA GCC
 E   Q   S   Q   A   T   I   A   E   I   E   A   K   E   K   K   F   K   E   A>

              1270          1280          1290          1300          1310          1320
CTC GAG GTG CTG CAG AAA GAA CAG GAG GTG CTG GCA GCG CTG GGC ATT GGC ACA ACC TCG
 L   E   V   L   Q   K   E   Q   E   V   L   A   A   L   G   I   G   T   T   S>

              1330          1340          1350          1360          1370          1380
CTC TAT TCC CAC AAC GGA TCA CCC TTA GCC CCC AAA AAC GCC AAT GAG AGA AGA CCC AGG
 L   Y   S   H   N   G   S   P   L   A   P   K   N   A   N   E   R   R   P   R>

              1390          1400          1410          1420          1430          1440
GTG AAA TCA AGG ATG TCA GAA GGC TCG ACA GAT GAC AAC AGA TCA CTA CAA TCC GAC CCT
 V   K   S   R   M   S   E   G   S   T   D   D   N   R   S   L   Q   S   D   P>

              1450          1460          1470          1480          1490          1500
TAC AAC CAG CGC AGG ATG TCT TTC CTA GGC CTT TCT TCT GGA AGA CGC AGG GCT AGC CAC
 Y   N   Q   R   R   M   S   F   L   G   L   S   S   G   R   R   R   A   S   H>

              1510          1520          1530          1540          1550          1560
AGC AGT GTG TTC CAC TTC CGA GCA CCC AGC CAA GAC GTC TCA TTT CCT GAT GGG ATC TTG
 S   S   V   F   H   F   R   A   P   S   Q   D   V   S   F   P   D   G   I   L>

              1570          1580          1590          1600          1610          1620
GAT GAC GGG GTC TTT CAT GGA GAT CAG GAA AGC CGT CGA AGT TCC ATA TTG CTG GGC AGG
 D   D   G   V   F   H   G   D   Q   E   S   R   R   S   I   L   L   G   R>

              1630          1640          1650          1660          1670          1680
GGT GCC GGG CAG GCA GGT CCT CTC CCC AGG AGT CCA CTG CCT CAG TCC CCC AAC CCT GGC
 G   A   G   Q   A   G   P   L   P   R   S   P   L   P   Q   S   P   N   P   G>

              1690          1700          1710          1720          1730          1740
CCT AGA CGT GGA GAA GAG GGA CAG CGT GGA GTG CCC ACT GGT GAG CTT GCC ACT GGA GCG
 P   R   R   G   E   E   G   Q   R   G   V   P   T   G   E   L   A   T   G   A>

              1750          1760          1770          1780          1790          1800
CCT GAA GGC CCG GCA CTC GAT GCT GCA GGA CAG AAG AAC TTC CTG TCT GCA GAC TAC TTG
 P   E   G   P   A   L   D   A   A   G   Q   K   N   F   L   S   A   D   Y   L>

              1810          1820          1830          1840          1850          1860
AAT GAA CCT TTC CGA GCA CAG AGG GCA ATG AGT GTT GTC AGT ATT ATG ACT TCT GTC ATT
 N   E   P   F   R   A   Q   R   A   M   S   V   V   S   I   M   T   S   V   I>

              1870          1880          1890          1900          1910          1920
GAG GAG CTG GAA GAG TCT AAG CTG AAG TGC CCA CCC TGC TTG ATC AGC TTA GCC CAG AAG
 E   E   L   E   E   S   K   L   K   C   P   P   C   L   I   S   L   A   Q   K>

              1930          1940          1950          1960          1970          1980
TAC CTG ATA TGG GAG TGC TGC CCC AAG TGG AAG AAA TTC AAG ATG GTG CTC TTC GAG CTG
 Y   L   I   W   E   C   C   P   K   W   K   K   F   K   M   V   L   F   E   L>

              1990          2000          2010          2020          2030          2040
GTG ACT GAC CCC TTC GCA GAG CTC ACC ATC ACC CTG TGC ATT GTG GTG AAT ACC GTC TTC
 V   T   D   P   F   A   E   L   T   I   T   L   C   I   V   V   N   T   V   F>

              2050          2060          2070          2080          2090          2100
ATG GCC ATG GAA CAC TAC CCC ATG ACT GAT GCC TTC GAT GCC ATG CTC CAA GCC GGC AAC
 M   A   M   E   H   Y   P   M   T   D   A   F   D   A   M   L   Q   A   G   N>
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          2110            2120            2130            2140            2150            2160
ATT GTC TTT ACT GTG TTT TTT ACA ATG GAG ATG GCC TTC AAG ATC ATT GCC TTC GAC CCC
 I   V   F   T   V   F   F   T   M   E   M   A   F   K   I   I   A   F   D   P>

          2170            2180            2190            2200            2210            2220
TAC TAC TAC TTC CAG AAG AAG TGG AAC ATC TTC GAC TGT GTC ATC GTC ACC GTG AGC CTG
 Y   Y   Y   F   Q   K   K   W   N   I   F   D   C   V   I   V   T   V   S   L>

          2230            2240            2250            2260            2270            2280
CTG GAG CTG AGC ACA TCC AAG AAG GGC AGC TTG TCT GTG CTC CGC ACC TTC CGC TTG CTT
 L   E   L   S   T   S   K   K   G   S   L   S   V   L   R   T   F   R   L   L>

          2290            2300            2310            2320            2330            2340
CGG GTC TTC AAG CTG GCC AAG TCC TGG CCC ACC CTG AAC ATG CTC ATC AAG ATC ATC GGG
 R   V   F   K   L   A   K   S   W   P   T   L   N   M   L   I   K   I   I   G>

          2350            2360            2370            2380            2390            2400
AAC TCT GTG GGG GCC CTG GGC AAC CTG ACC TTC ATC CTG GCC ATC ATC GTC TTT ATC TTC
 N   S   V   G   A   L   G   N   L   T   F   I   L   A   I   I   V   F   I   F>

          2410            2420            2430            2440            2450            2460
GCC CTG GTG GGA AAG CAG CTC CTC TCA GAG AAC TAT GGG TGC CGC AGG GAT GGC ATC TCC
 A   L   V   G   K   Q   L   L   S   E   N   Y   G   C   R   R   D   G   I   S>

          2470            2480            2490            2500            2510            2520
GTG TGG AAT GGT GAG AGG CTG CGC TGG CAC ATG TGT GAC TTC TTC CAT TCC TTC CTC GTC
 V   W   N   G   E   R   L   R   W   H   M   C   D   F   F   H   S   F   L   V>

          2530            2540            2550            2560            2570            2580
GTC TTC CGG ATC CTC TGC GGG GAG TGG ATC GAG AAC ATG TGG GTC TGC ATG GAG GTC AGC
 V   F   R   I   L   C   G   E   W   I   E   N   M   W   V   C   M   E   V   S>

          2590            2600            2610            2620            2630            2640
CAG GAC TAC ATC TGC CTC ACC CTC TTC TTG ACA GTG ATG GTG CTA GGC AAC CTG GTG GTG
 Q   D   Y   I   C   L   T   L   F   L   T   V   M   V   L   G   N   L   V   V>

          2650            2660            2670            2680            2690            2700
CTC AAC CTA TTC ATC GCT TTA CTG CTG AAC TCC TTC AGT GCG GAC AAC CTC ACA GCC CCA
 L   N   L   F   I   A   L   L   L   N   S   F   S   A   D   N   L   T   A   P>

          2710            2720            2730            2740            2750            2760
GAG GAT GAC GGG GAG GTG AAC AAC TTG CAG GTA GCA CTG GCC CGG ATT CAG GTA TTT GGC
 E   D   D   G   E   V   N   N   L   Q   V   A   L   A   R   I   Q   V   F   G>

          2770            2780            2790            2800            2810            2820
CAT CGG GCC AGT CGG GCC ATT ACC AGT TAC ATC AGA AGC CAC TGC CGG CTC CGC TGG CCC
 H   R   A   S   R   A   I   T   S   Y   I   R   S   H   C   R   L   R   W   P>

          2830            2840            2850            2860            2870            2880
AAG GTG GAG ACC CAG CTG GGG ATG AAA CCC CCA CTC ACC AGC TGC AAA GCT GAG AAC CAC
 K   V   E   T   Q   L   G   M   K   P   P   L   T   S   C   K   A   E   N   H>

          2890            2900            2910            2920            2930            2940
ATT GCT ACT GAT GCT GNC AAT GCT GCA GTG GGG AAC CAG GCA AAG CCA GCT CTT GGT GGC
 I   A   T   D   A   X   N   A   A   V   G   N   Q   A   K   P   A   L   G   G>

          2950            2960            2970            2980            2990            3000
CCC AAG GAG AAC CAC GGG GAC TTC ATC ACT GAT CCT AAC GTG TGG GTC TCT GTG CCC ATT
 P   K   E   N   H   G   D   F   I   T   D   P   N   V   W   V   S   V   P   I>

          3010            3020            3030            3040            3050            3060
GCT GAG GGG GAG TCC GAC CTT GAT GAG CTC GAG GAA GAT GTG GAG CAT GCT TCT CAG AGC
 A   E   G   E   S   D   L   D   E   L   E   E   D   V   E   H   A   S   Q   S>

          3070            3080            3090            3100            3110            3120
TCC TGG CAG GAA GAG AGC CCC AAA GGG CAG GAG CTG CTG CAG CAA GTC CAA AAG TGT GAA
 S   W   Q   E   E   S   P   K   G   Q   E   L   L   Q   Q   V   Q   K   C   E>

          3130            3140            3150            3160            3170            3180
GAT CAC CAG GCA GCC CGA AGC CCA CCC TCC GGG ATG TCC TCT GAG GAC CTG GCT CCA TAC
 D   H   Q   A   A   R   S   P   P   S   G   M   S   S   E   D   L   A   P   Y>
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              3190            3200            3210            3220            3230            3240
CTG GGG GAG AGA TGG CAG AGG GAG GAG AGC CCT CGG GTC CCT GCC GAG GGA GTG GAT GAC
 L   G   E   R   W   Q   R   E   E   S   P   R   V   P   A   E   G   V   D   D>

              3250            3260            3270            3280            3290            3300
ACA AGC TCC TCC GAG GGC AGC ACG GTG GAC TGC CCG GAC CCA GAG GAG ATC CTG AGG AAG
 T   S   S   S   E   G   S   T   V   D   C   P   D   P   E   E   I   L   R   K>

              3310            3320            3330            3340            3350            3360
ATC CCT GAG CTG GCG GAG GAG CTG GAC GAG CCC GAT GAC TGT TTC CCA GAA GGC TGC ACT
 I   P   E   L   A   E   E   L   D   E   P   D   D   C   F   P   E   G   C   T>

              3370            3380            3390            3400            3410            3420
CGC CGC TGT CCC TGC TGC AAA GTG AAC ACC AGT AAG TTT CCT TGG GCC ACG GGC TGG CAG
 R   R   C   P   C   C   K   V   N   T   S   K   F   P   W   A   T   G   W   Q>

              3430            3440            3450            3460            3470            3480
GTG CGC AAA ACC TGT TAC CGC ATT GTG GAG CAC AGC TGG TTT GAG AGT TTC ATT ATC TTC
 V   R   K   T   C   Y   R   I   V   E   H   S   W   F   E   S   F   I   I   F>

              3490            3500            3510            3520            3530            3540
ATG ATC CTG CTC AGC AGT GGA GCG CTG GCC TTT GAG GAT AAC TAC CTG GAA GAA AAG CCC
 M   I   L   L   S   S   G   A   L   A   F   E   D   N   Y   L   E   E   K   P>

              3550            3560            3570            3580            3590            3600
CGA GTG AAG TCT GTG CTG GAG TAC ACT GAC AGA GTG TTC ACT TTC ATC TTT GTA TTC GAG
 R   V   K   S   V   L   E   Y   T   D   R   V   F   T   F   I   F   V   F   E>

              3610            3620            3630            3640            3650            3660
ATG TTG CTC AAG TGG GTA GCT TAT GGC TTC AAA AAA TAT TTC ACC AAT GCC TGG TGC TGG
 M   L   L   K   W   V   A   Y   G   F   K   K   Y   F   T   N   A   W   C   W>

              3670            3680            3690            3700            3710            3720
CTG GAC TTC CTC ATC GTG AAT ATC TCC CTC ACA AGC CTC ATA GCC AAG ATC CTC GAG TAT
 L   D   F   L   I   V   N   I   S   L   T   S   L   I   A   K   I   L   E   Y>

              3730            3740            3750            3760            3770            3780
TCA GAC GTG GCG TCC ATC AAA GCC CTT CGG ACT CTC CGT GCC CTC CGG CCG CTG CGG GCT
 S   D   V   A   S   I   K   A   L   R   T   L   R   A   L   R   P   L   R   A>

              3790            3800            3810            3820            3830            3840
CTG TCT CGA TTC GAA GGC ATG AGG GTA GTG GTG GAT GCC TTG GTG GGC GCC ATT CCC TCC
 L   S   R   F   E   G   M   R   V   V   V   D   A   L   V   G   A   I   P   S>

              3850            3860            3870            3880            3890            3900
ATC ATG AAC GTC CTC CTC GTC TGC CTC ATC TTC TGG CTC ATC TTC AGC ATC ATG GGT GTG
 I   M   N   V   L   L   V   C   L   I   F   W   L   I   F   S   I   M   G   V>

              3910            3920            3930            3940            3950            3960
AAC CTC TTC GCC GGG AAA TTT TCG AGA TGT GTC GAC ACC AGA AGC AAC CCA TTT TCC GTC
 N   L   F   A   G   K   F   S   R   C   V   D   T   R   S   N   P   F   S   V>

              3970            3980            3990            4000            4010            4020
GTG AAT TCG ACA TTC GTG ACT AAC AAG TCT GAC TGT TAC AAT CAA AAC AAT ACT GGC CAC
 V   N   S   T   F   V   T   N   K   S   D   C   Y   N   Q   N   N   T   G   H>

              4030            4040            4050            4060            4070            4080
TTC TTC TGG GTT AAC GTC AAA GTC AAC TTC GAC AAC GTT GCT ATG GGC TAC CTC GCG CTT
 F   F   W   V   N   V   K   V   N   F   D   N   V   A   M   G   Y   L   A   L>

              4090            4100            4110            4120            4130            4140
CTC CAG GTG GCA ACC TTC AAA GGC TGG ATG GAC ATT ATG TAT GCA GCT GTC GAT TCT CGA
 L   Q   V   A   T   F   K   G   W   M   D   I   M   Y   A   A   V   D   S   R>

              4150            4160            4170            4180            4190            4200
GAT ATC AAC AGT CAG CCC AAT TGG GAG GAG AGC CTG TAC ATG TAC CTA TAC TTC GTC GTC
 D   I   N   S   Q   P   N   W   E   E   S   L   Y   M   Y   L   Y   F   V   V>

              4210            4220            4230            4240            4250            4260
TTC ATC ATT TTC GGT GGC TTC TTC ACG CTG AAT CTC TTT GTC GGG GTC ATC ATT GAC AAC
 F   I   I   F   G   G   F   F   T   L   N   L   F   V   G   V   I   I   D   N>
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          4270           4280           4290           4300           4310           4320
TTC AAT CAA CAG AAA AAA AAG CTA GGG GGC CAG GAC ATC TTC ATG ACA GAG GAG CAG AAG
 F   N   Q   Q   K   K   K   L   G   G   Q   D   I   F   M   T   E   E   Q   K>

          4330           4340           4350           4360           4370           4380
AAG TAC TAC AAT GCC ATG AAG AAG CTG GGC TCC AAG AAA CCC CAG AAG CCC ATC CCA CGG
 K   Y   Y   N   A   M   K   K   L   G   S   K   K   P   Q   K   P   I   P   R>

          4390           4400           4410           4420           4430           4440
CCT TTG AAT AAG TAC CAG GGC TTC GTG TTT GAC ATT GTG ACC AGG CAA GCA TTT GAC ATC
 P   L   N   K   Y   Q   G   F   V   F   D   I   V   T   R   Q   A   F   D   I>

          4450           4460           4470           4480           4490           4500
ATC ATC ATG GCT CTC ATC TGC CTC AAC ATG ATC ACC ATG ATG GTG GAG ACC GAC AAT CAG
 I   I   M   A   L   I   C   L   N   M   I   T   M   M   V   E   T   D   N   Q>

          4510           4520           4530           4540           4550           4560
AGC GAG GAG AAG ACG AAG GTC CTG GGC AGA ATC AAC CAG TTC TTC GTG GCC GTC TTC ACG
 S   E   E   K   T   K   V   L   G   R   I   N   Q   F   F   V   A   V   F   T>

          4570           4580           4590           4600           4610           4620
GGC GAG TGT GTG ATG AAG ATG TTC GCC CTT CGG CAG TAT TAC TTC ACC AAC GGC TGG AAT
 G   E   C   V   M   K   M   F   A   L   R   Q   Y   Y   F   T   N   G   W   N>

          4630           4640           4650           4660           4670           4680
GTG TTC GAC TTC ATT GTG GTG ATT CTG TCC ATT TCT AGT CTG TTG TTT TCT GCG ATC CTT
 V   F   D   F   I   V   V   I   L   S   I   S   S   L   L   F   S   A   I   L>

          4690           4700           4710           4720           4730           4740
AGC TCA CTA GAA AGT TAC TTC TCC CCC ACG CTC TTA CGC GTC ATC CGT CTG GCC AGG ATC
 S   S   L   E   S   Y   F   S   P   T   L   L   R   V   I   R   L   A   R   I>

          4750           4760           4770           4780           4790           4800
GGC CGC ATC CTC AGG CTG ATT CGA GCA GCC AAG GGG ATT CGC ACG CTG CTC TTC GCC CTC
 G   R   I   L   R   L   I   R   A   A   K   G   I   R   T   L   L   F   A   L>

          4810           4820           4830           4840           4850           4860
ATG ATG TCC CTG CCC GCC CTC TTC AAC ATC GGC CTC CTC CTC TTC CTC GTC ATG TTC ATC
 M   M   S   L   P   A   L   F   N   I   G   L   L   L   F   L   V   M   F   I>

          4870           4880           4890           4900           4910           4920
TAC TCC ATC TTC GGC ATG ACC AGC TTC GCT AAT GTC ATA GAT GAG GCT GGC ATC GAC GAC
 Y   S   I   F   G   M   T   S   F   A   N   V   I   D   E   A   G   I   D   D>

          4930           4940           4950           4960           4970           4980
ATG TTC AAC TTC AAG ACC TTT GGC AAC AGC ATG CTG TGC CTT TTC CAG ATC ACC ACG TCG
 M   F   N   F   K   T   F   G   N   S   M   L   C   L   F   Q   I   T   T   S>

          4990           5000           5010           5020           5030           5040
GCT GGC TGG GAT GGC CTC CTC AGC CCC ATC CTC AAC ACA GGA CCC CCC TAC TGC GAC CCC
 A   G   W   D   G   L   L   S   P   I   L   N   T   G   P   P   Y   C   D   P>

          5050           5060           5070           5080           5090           5100
AAC CGG CCC AAC AGC AAT GGC TCC AAG GGG AAT TGT GGA AGC CCA GCG GTG GGC ATC CTC
 N   R   P   N   S   N   G   S   K   G   N   C   G   S   P   A   V   G   I   L>

          5110           5120           5130           5140           5150           5160
TTC TTC ACC ACC TAC ATC ATC ATC TCC TTC CTC ATC GTG GTC AAC ATG TAC ATT GCA GTG
 F   F   T   T   Y   I   I   I   S   F   L   I   V   V   N   M   Y   I   A   V>

          5170           5180           5190           5200           5210           5220
ATT CTG GAG AAC TTC AAT GTG GCC ACA GAA GAG AGC ACG GAG CCC CTG AGC GAG GAC GAC
 I   L   E   N   F   N   V   A   T   E   E   S   T   E   P   L   S   E   D   D>

          5230           5240           5250           5260           5270           5280
TTT GAC ATG TTC TAT GAG ACC TGG GAG AAG TTT GAC CCG GAG GCC ACC CAG TTC ATT GCC
 F   D   M   F   Y   E   T   W   E   K   F   D   P   E   A   T   Q   F   I   A>

          5290           5300           5310           5320           5330           5340
TTT TCT GCC CTC TCA GAC TTT GCA GAC ACA CTC TCT GGC CCT CTT AGA ATC CCA AAA CCT
 F   S   A   L   S   D   F   A   D   T   L   S   G   P   L   R   I   P   K   P>
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        5350        5360        5370        5380        5390        5400
AAT CAG AAT ATA TTA ATC CAG ATG GAC CTG CCG TTG GTC CCC GGA GAT AAG ATC CAC TGT
 N   Q   N   I   L   I   Q   M   D   L   P   L   V   P   G   D   K   I   H   C>

        5410        5420        5430        5440        5450        5460
TTG GAC ATC CTC TTT GCC TTC ACA AAG AAT GTC TTG GGA GAA TCT GGG GAG TTG GAT TCT
 L   D   I   L   F   A   F   T   K   N   V   L   G   E   S   G   E   L   D   S>

        5470        5480        5490        5500        5510        5520
CTG AAG ACT AAT ATG GAA GAG AAG TTT ATG GCA ACT AAT CTT TCC AAA GCA TCC TAT GAA
 L   K   T   N   M   E   E   K   F   M   A   T   N   L   S   K   A   S   Y   E>

        5530        5540        5550        5560        5570        5580
CCA ATA GCA ACC ACC CTC CGG TGC AAG CAG GAA GAC ATC TCA GCC ACC ATT ATT CAA AAG
 P   I   A   T   T   L   R   C   K   Q   E   D   I   S   A   T   I   I   Q   K>

        5590        5600        5610        5620        5630        5640
GCC TAT CGG AAC TAC ATG TTG CAA CGC TCC TTG ATG CTC TCC AAC CCC CTG CAT GTG CCC
 A   Y   R   N   Y   M   L   Q   R   S   L   M   L   S   N   P   L   H   V   P>

        5650        5660        5670        5680        5690        5700
AGG GCT GAG GAG GAT GGC GTG TCA CTC CCC AGG GAA GGC TAT GTT ACA TTC ATG GCA AAT
 R   A   E   E   D   G   V   S   L   P   R   E   G   Y   V   T   F   M   A   N>

        5710        5720        5730        5740        5750        5760
GAC AAC GGT GGG CTC CCA GAC AAA TCG GAA ACT GCT TCT GCT ACG TCT TTC CCA CCA TCC
 D   N   G   G   L   P   D   K   S   E   T   A   S   A   T   S   F   P   P   S>

        5770        5780        5790        5800        5810        5820
TAT GAC AGC GTC ACC AGG GGC CTG AGT GAC AGG GCC AAC ATT AGC ACA TCT AGC TCA ATG
 Y   D   S   V   T   R   G   L   S   D   R   A   N   I   S   T   S   S   S   M>

        5830        5840        5850        5860        5870
CAA AAT GAA GAT GAA GTC ACT GCT AAG GAA GGG AAG AGC CCT GGA CCT CAG TGA
 Q   N   E   D   E   V   T   A   K   E   G   K   S   P   G   P   Q   *>
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Appendix B

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                      10              20              30              40
Y09108-cds      A T G G A G T T C C C C T T T G G G T C C G T G G G A A C T A C C A A C T T C A
mScn10a cds (GRI) A T G G A G T T C C C C T T T G G G T C C G T G G G A A C T A C C A A C T T C A


                      50              60              70              80
Y09108-cds      G A C G G T T C A C T C C A G A G T C G C T G G C A G A G A T C G A G A A G C A
mScn10a cds (GRI) G A C G G T T C A C T C C A G G G T C G C T G G C A G A G A T C G A G A A G C A


                      90             100             110             120
Y09108-cds      G A T C G C T G C C C A C C G C G C C G C C A A G A A G G G C A G A A C T A A G
mScn10a cds (GRI) G A T C G C T G C C C A C C G C G C C G C C A A G A A G G G C A G A C C T A A G


                     130             140             150             160
Y09108-cds      C A A A G A G G A C A G A A G G A C A A G A G T G A G A A G C C C A G G C C T C
mScn10a cds (GRI) C A A A G A G G A C A G A A G G A C A A G A G T G A G A A G C C C A G G C C T C


                     170             180             190             200
Y09108-cds      A G T T G G A C T T G A A G G C C T G T A A C C A G C T G C C C A G G T T C T A
mScn10a cds (GRI) A G T T G G A C T T G A A G G C C T G T A A C C A G C T G C C C A G G T T C T A


                     210             220             230             240
Y09108-cds      T G G C G A G C T C C C A G C A G A G C T G G T C G G G G A G C C C C T G G A G
mScn10a cds (GRI) T G G C G A G C T C C C A G C A G A G C T G G T C G G G G A G C C C C T G G A G


                     250             260             270             280
Y09108-cds      G A C C T G G A T C C T T T T T A C A G C A C A C A C A G G A C A T T C A T A C
mScn10a cds (GRI) G A C C T G G A T C C T T T C T A C A G C A C A C A C C G G A C A T T C A T A G


                     290             300             310             320
Y09108-cds      T G T T G A A T A A A A G C A G G A C C A T T T C C A G A T T C A G T G C C A C
mScn10a cds (GRI) T G T T G G A T A A A A G C A G G A C C A T T T C C A G A T T C A G T G C C A C


                     330             340             350             360
Y09108-cds      T T G G G C T C T G T G G C T C T T C A G T C C C T T C A A C C T G A T C A G A
mScn10a cds (GRI) T T G G G C T C T G T G G C T C T T C A G T C C C T T C A A C C T G A T C A G A


                     370             380             390             400
Y09108-cds      A G A A C A G C C A T C A A A G T G T C C G T C C A C T C C T G G T T C T C C A
mScn10a cds (GRI) A G A A C A G C C A T C A A A G T G T C C G T C C A C T C C T G G T T C T C C A


                     410             420             430             440
Y09108-cds      T A T T T A T C A C T G T C A C T A T T T T G G T C A A C T G T G T G T G C A T
mScn10a cds (GRI) T A T T T A T C A C T G T C A C T A T T T T G G T C A A C T G T G T G T G C A T
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                         490              500              510              520
Y09108-cds       A C T G T T G T T T A C A C C T T C G A G G C T C T G A T A A A G A T A C T G G
mScn10a cds (GRI) A C T G T T G T T T A C A C C T T C G A G G C T C T G A T A A A G A T A C T G G


                         530              540              550              560
Y09108-cds       C A A G A G G G T T T T G T C T T A A T G A A T T C A C T T A T C T T C G A G A
mScn10a cds (GRI) C A A G A G G G T T T T G T C T A A A T G A A T T C A C T T A T C T T C G A G A


                         570              580              590              600
Y09108-cds       T C C C T G G A A C T G G C T G G A C T T C A G T G T C A T T A C C T T G G C A
mScn10a cds (GRI) T C C C T G G A A C T G G C T G G A C T T C A G T G T C A T T A C C C T G G C G


                         610              620              630              640
Y09108-cds       T A C G T G G G T G C A G C G G T A G A C C T C C G A G G A A T C T C A G G C C
mScn10a cds (GRI) T A T G T G G G T G C A G C G A T A G A C C T C C G A G G A A T C T C A G G C C


                         650              660              670              680
Y09108-cds       T G C G G A C A T T C C G A G T T C T C A G G G C C C T G A A G A C T G T T T C
mScn10a cds (GRI) T G C G G A C A T T C C G A G T T C T C A G G G C C C T G A A G A C T G T T T C


                         690              700              710              720
Y09108-cds       T G T G A T C C C A G G A C T G A A G G T C A T C G T G G G A G C C C T G A T C
mScn10a cds (GRI) T G T G A T C C C A G G A C T G A A G G T C A T C G T G G G A G C C C T G A T C


                         730              740              750              760
Y09108-cds       C A C T C A G T G A G G A A G C T G G C C G A C G T G A C C A T C C T C A C A G
mScn10a cds (GRI) C A C T C A G T G A G G A A G C T G G C C G A C G T G A C C A T C C T C A C A G


                         770              780              790              800
Y09108-cds       T C T T C T G C C T G A G T G T C T T T G C C T T G G T G G G C C T G C A G C T
mScn10a cds (GRI) T C T T C T G C C T G A G T G T C T T T G C C T T G G T G G G C C T G C A G C T


                         810              820              830              840
Y09108-cds       C T T C A A G G G G A A C C T C A A G A A T A A A T G C A T T A A G A A C G G C
mScn10a cds (GRI) C T T C A A G G G G A A C C T C A A G A A T A A A T G C A T T A A G A A C G G C


                         850              860              870              880
Y09108-cds       A C A G A T C C G C A C A A G G C T G A C A A T C T C T C A T C T G A A A T G G
mScn10a cds (GRI) A C A G A T C C G C A C A A G G C T G A C A A T C T C T C A T C T G A A A T G G


                         890              900              910              920
Y09108-cds       C A G A A G A C A T C T T C A T C A A G C C C G G T A C T A C G G A T C C C T T
mScn10a cds (GRI) C A G G A G A C A T C T T C A T C A A G C C C G G T A C T A C G G A T C C C T T


                         930              940              950              960
Y09108-cds       G T T G T G T G G C A A T G G G T C T G A T G C T G G C C A C T G C C C T A A T
mScn10a cds (GRI) G T T G T G T G G C A A T G G A T C T G A T G C T G G C C A C T G C C C T A A T
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                          1010              1020              1030              1040
Y09108-cds        A C T A C A C C A G C T T T G A T T C C T T T G C G T G G G C G T T C C T C T C
mScn10a cds (GRI) A C T A C A C C A G C T T T G A T T C C T T C G C G T G G G C G T T C C T C T C


                          1050              1060              1070              1080
Y09108-cds        A C T G T T C C G T C T C A T G A C G C A G G A C T C C T G G G A A C G G C T G
mScn10a cds (GRI) A C T G T T C C G T C T C A T G A C G C A G G A C T C C T G G G A A C G G C T G


                          1090              1100              1110              1120
Y09108-cds        T A C C A G C A G A C A C T C C G G G C T T C C G G G A A A A T G T A C A T G G
mScn10a cds (GRI) T A C C A G C A G A C A C T C C G G G C T T C C G G G A A A A T G T A C A T G G


                          1130              1140              1150              1160
Y09108-cds        T C T T T T T T G T G C T G G T C A T T T T C C T T G G A T C A T T C T A C C T
mScn10a cds (GRI) T C T T T T T T G T G C T G G T C A T C T T C C T T G G A T C A T T C T A C C T


                          1170              1180              1190              1200
Y09108-cds        G G T C A A T T T G A T C T T G G C T G T G G T C A C C A T G G C A T A T G A G
mScn10a cds (GRI) G G T C A A T T T G A T C T T G G C T G T G G T C A C C A T G G C A T A T G A G


                          1210              1220              1230              1240
Y09108-cds        G A A C A G A G C C A G G C A A C A A T T G C A G A A A T C G A A G C C A A G G
mScn10a cds (GRI) G A A C A G A G C C A G G C A A C A A T T G C A G A A A T C G A A G C C A A G G


                          1250              1260              1270              1280
Y09108-cds        A A A A A A A G T T C C A G G A A G C C C T C G A G G T G C T G C A A A A A G A
mScn10a cds (GRI) A G A A G A A G T T C A A G G A A G C C C T C G A G G T G C T G C A G A A A G A


                          1290              1300              1310              1320
Y09108-cds        A C A G G A G G T G T T G G C A G C G C T G G G A A T T G A C A C A A C T T C G
mScn10a cds (GRI) A C A G G A G G T G C T G G C A G C G C T G G G C A T T G G C A C A A C C T C G


                          1330              1340              1350              1360
Y09108-cds        T T T T A T T C C C A C A G C G G A T C A C C C T T A G C C T C C A A A A A C G
mScn10a cds (GRI) C T C T A T T C C C A C A A C G G A T C A C C C T T A G C C C C C A A A A A C G


                          1370              1380              1390              1400
Y09108-cds        C C A A T G A G A G A A G A C C C A G G G T G A A A T C A A G G G T G T C A G A
mScn10a cds (GRI) C C A A T G A G A G A A G A C C C A G G G T G A A A T C A A G G A T G T C A G A


                          1410              1420              1430              1440
Y09108-cds        A G G C T C C A C A G A T G A C A A C A G A T C A C C A C A A T C C G A C C C T
mScn10a cds (GRI) A G G C T C G A C A G A T G A C A A C A G A T C A C T A C A A T C C G A C C C T


                          1450              1460              1470              1480
Y09108-cds        T A C A A C C A G C G C A G G A T G T C C T T C C T A G G C C T T T C T T C T G
mScn10a cds (GRI) T A C A A C C A G C G C A G G A T G T C T T T C C T A G G C C T T T C T T C T G
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                                    1530            1540            1550            1560
Y09108-cds        A G C A C C C A G C C A A G A C G T C T C A T T T C C T G A T G G G A T C T T G
mScn10a cds (GRI) A G C A C C C A G C C A A G A C G T C T C A T T T C C T G A T G G G A T C T T G


                                    1570            1580            1590            1600
Y09108-cds        G A C G A T G G G G T C T T T C A T G G A G A T C A G G A A A G C C G T C G A A
mScn10a cds (GRI) G A T G A C G G G G T C T T T C A T G G A G A T C A G G A A A G C C G T C G A A


                                    1610            1620            1630            1640
Y09108-cds        A T T C C A T A T T G C T G G G C A G G G G T G C C G G C C A G G C A G G T C C
mScn10a cds (GRI) G T T C C A T A T T G C T G G G C A G G G G T G C C G G G C A G G C A G G T C C


                                    1650            1660            1670            1680
Y09108-cds        T C T C C C C A G A A G T C C A C T G C C T C A G T C C C C C A A C C C T G G C
mScn10a cds (GRI) T C T C C C C A G G A G T C C A C T G C C T C A G T C C C C C A A C C C T G G C


                                    1690            1700            1710            1720
Y09108-cds        C G T A A A C A T G G A A A A G A G G G A C A G C T T G G A A T G C C C A C T G
mScn10a cds (GRI) C C T A G A C G T G G A G A A G A G G G A C A G C G T G G A G T G C C C A C T G


                                    1730            1740            1750            1760
Y09108-cds        G T G A A C T T G C C G C T G G A A C G C C T G A A G G C C C G G C A C T C G A
mScn10a cds (GRI) G T G A G C T T G C C A C T G G A G C G C C T G A A G G C C C G G C A C T C G A


                                    1770            1780            1790            1800
Y09108-cds        T G C T G C A G G A C A G A A G A A C T T C C T G T C T G C A G G C T A C T T G
mScn10a cds (GRI) T G C T G C A G G A C A G A A G A A C T T C C T G T C T G C A G A C T A C T T G


                                    1810            1820            1830            1840
Y09108-cds        A A T G A A C C T T T C C G A G C A C A G A G G G C A A T G A G T G T T G T C A
mScn10a cds (GRI) A A T G A A C C T T T C C G A G C A C A G A G G G C A A T G A G T G T T G T C A


                                    1850            1860            1870            1880
Y09108-cds        G T A T C A T G A C T T C T G T C A T T G A G G A A C T G G A A G A A T C T A A
mScn10a cds (GRI) G T A T T A T G A C T T C T G T C A T T G A G G A G C T G G A A G A G T C T A A


                                    1890            1900            1910            1920
Y09108-cds        G C T G A A G T G C C C A C C C T G C T T G A T C A G C T T C G C C C A A A A A
mScn10a cds (GRI) G C T G A A G T G C C C A C C C T G C T T G A T C A G C T T A G C C C A G A A G


                                    1930            1940            1950            1960
Y09108-cds        T A T C T G A T A T G G G A A T G C T G C C C C A A G T G G A G A A A A T T C A
mScn10a cds (GRI) T A C C T G A T A T G G G A G T G C T G C C C C A A G T G G A A G A A A A T T C A


                                    1970            1980            1990            2000
Y09108-cds        A A A T G G T G C T C C T C G A A C T G G T G A C T G A C C C C T T C G C A G A
mScn10a cds (GRI) A G A T G G T G C T C T T C G A G C T G G T G A C T G A C C C C T T C G C A G A
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                             2050              2060              2070              2080
Y09108-cds        A T G G C C A T G G A A C A C T A C C C C A T G A C T G A T G C T T T C G A T G
mScn10a cds (GRI) A T G G C C A T G G A A C A C T A C C C C A T G A C T G A T G C C T T C G A T G


                             2090              2100              2110              2120
Y09108-cds        C C A T G C T C C A A G C C G G C A A C A T T G T C T T C A C T G T G T T T T T
mScn10a cds (GRI) C C A T G C T C C A A G C C G G C A A C A T T G T C T T T A C T G T G T T T T T


                             2130              2140              2150              2160
Y09108-cds        T A C A A T G G A G A T G G C C T T C A A G A T C A T T G C T T T C G A C C C G
mScn10a cds (GRI) T A C A A T G G A G A T G G C C T T C A A G A T C A T T G C C T T C G A C C C C


                             2170              2180              2190              2200
Y09108-cds        T A C T A C T A C T T C C A G A A G A A G T G G A A C A T C T T C G A C T G T G
mScn10a cds (GRI) T A C T A C T A C T T C C A G A A G A A G T G G A A C A T C T T C G A C T G T G


                             2210              2220              2230              2240
Y09108-cds        T C A T T G T C A C C G T G A G C C T G C T G G A G C T G A G T G C A T C C A A
mScn10a cds (GRI) T C A T C G T C A C C G T G A G C C T G C T G G A G C T G A G C A C A T C C A A


                             2250              2260              2270              2280
Y09108-cds        A A A G G G C A G C C T A T C T G T G C T C C G T T C C T T A C G C T T G C T T
mScn10a cds (GRI) G A A G G G C A G C T T G T C T G T G C T C C G C A C C T T C C G C T T G C T T


                             2290              2300              2310              2320
Y09108-cds        C G G G T C T T C A A G C T G G C C A A G T C C T G G C C C A C C C T G A A C A
mScn10a cds (GRI) C G G G T C T T C A A G C T G G C C A A G T C C T G G C C C A C C C T G A A C A


                             2330              2340              2350              2360
Y09108-cds        T G C T C A T C A A G A T C A T C G G G A A C T C T G T G G G G G C C C T G G G
mScn10a cds (GRI) T G C T C A T C A A G A T C A T C G G G A A C T C T G T G G G G G C C C T G G G


                             2370              2380              2390              2400
Y09108-cds        C A A C C T G A C C T T C A T C C T G G C C A T C A T C G T C T T C A T C T T T
mScn10a cds (GRI) C A A C C T G A C C T T C A T C C T G G C C A T C A T C G T C T T T A T C T T C


                             2410              2420              2430              2440
Y09108-cds        G C C C T G G T G G G A A A G C A G C T C C T C T C A G A G A A C T A T G G G T
mScn10a cds (GRI) G C C C T G G T G G G A A A G C A G C T C C T C T C A G A G A A C T A T G G G T


                             2450              2460              2470              2480
Y09108-cds        G C C G C A G G G A T G G C G T C T C C G T G T G G A A T G G T G A G A A G C T
mScn10a cds (GRI) G C C G C A G G G A T G G C A T C T C C G T G T G G A A T G G T G A G A G G C T


                             2490              2500              2510              2520
Y09108-cds        G C G C T G G C A C A T G T G T G A C T T C T T C C A T T C C T T C C T C G T C
mScn10a cds (GRI) G C G C T G G C A C A T G T G T G A C T T C T T C C A T T C C T T C C T C G T C
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                            2570              2580              2590              2600
Y09108-cds      G G G T C T G C A T G G A G G T C A G C C A G A A C T A C A T C T G C C T C A C
mScn10a cds (GRI) G G G T C T G C A T G G A G G T C A G C C A G G A C T A C A T C T G C C T C A C


                            2610              2620              2630              2640
Y09108-cds      C C T C T T C T T G A C A G T G A T G G T G C T A G G C A A C C T G G T G G T G
mScn10a cds (GRI) C C T C T T C T T G A C A G T G A T G G T G C T A G G C A A C C T G G T G G T G


                            2650              2660              2670              2680
Y09108-cds      C T C A A C C T T T T T A T C G C T T T A C T G C T G A A T T C C T T T A G T G
mScn10a cds (GRI) C T C A A C C T A T T C A T C G C T T T A C T G C T G A A C T C C T T C A G T G


                            2690              2700              2710              2720
Y09108-cds      C G G A C A A C C T C A C G G C C C C A G A G G A T G A C G G G G A G G T G A A
mScn10a cds (GRI) C G G A C A A C C T C A C A G C C C C A G A G G A T G A C G G G G A G G T G A A


                            2730              2740              2750              2760
Y09108-cds      C A A C T T G C A G T T A G C A C T G G C C A G G A T T C A G G T A C T T G G C
mScn10a cds (GRI) C A A C T T G C A G G T A G C A C T G G C C C G G A T T C A G G T A T T T G G C


                            2770              2780              2790              2800
Y09108-cds      C A T C G G G C C A G T C G G G C C A T T A C C A G T T A C A T C A G A A G C C
mScn10a cds (GRI) C A T C G G G C C A G T C G G G C C A T T A C C A G T T A C A T C A G A A G C C


                            2810              2820              2830              2840
Y09108-cds      A T T G C C G G T T C C G T T G G C C C A A G G T G G A G A C C C A G C T G G G
mScn10a cds (GRI) A C T G C C G G C T C C G C T G G C C C A A G G T G G A G A C C C A G C T G G G


                            2850              2860              2870              2880
Y09108-cds      G A T G A A A C C C C C A C T C A C C A G C T G C A A A G T T G A G A A C C A C
mScn10a cds (GRI) G A T G A A A C C C C C A C T C A C C A G C T G C A A A G C T G A G A A C C A C


                            2890              2900              2910              2920
Y09108-cds      A T T G C T A C T G A T G C T G T C A A T G C T G C A G T G G G G A A C C T G A
mScn10a cds (GRI) A T T G C T A C T G A T G C T G N C A A T G C T G C A G T G G G G A A C C A G G


                            2930              2940              2950              2960
Y09108-cds      C A A A G C C A G C T C T T A G T G G C C C C A A G G A G A A T C A C G G G G A
mScn10a cds (GRI) C A A A G C C A G C T C T T G G T G G C C C C A A G G A G A A C C A C G G G G A


                            2970              2980              2990              3000
Y09108-cds      C T T C A T C A C T G A T C C T A A C G T G T G G G T C T C T G T G C C C A T T
mScn10a cds (GRI) C T T C A T C A C T G A T C C T A A C G T G T G G G T C T C T G T G C C C A T T


                            3010              3020              3030              3040
Y09108-cds      G C T G A G G G G G A G T C C G A C C T T G A T G A G C T C G A G G A A G A T G
mScn10a cds (GRI) G C T G A G G G G G A G T C C G A C C T T G A T G A G C T C G A G G A A G A T G
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                               3090              3100              3110              3120
Y09108-cds        │C A A A G G G C A G│C A G│G A G C T G C T G C│C A│C A A G T C C A A A A G T G T│
mScn10a cds (GRI) │C A A A G G G C A G│N N N│G A G C T G C T G C│A G│C A A G T C C A A A A G T G T│


                               3130              3140              3150              3160
Y09108-cds        │G A A│A│A T C A C C A G G C A G C C│A│G A A G C C C A│A│C C T C C G G G A T G T│
mScn10a cds (GRI) │G A A│G│A T C A C C A G G C A G C C│C│G A A G C C C A│C│C C T C C G G G A T G T│


                               3170              3180              3190              3200
Y09108-cds        │C C T C T G A│A│G A C C T G G C T C C A T A C C T G G G G G A G A G A T G G│A│A│
mScn10a cds (GRI) │C C T C T G A│G│G A C C T G G C T C C A T A C C T G G G G G A G A G A T G G│C│A│


                               3210              3220              3230              3240
Y09108-cds        A│A G G│A│A G G A│T│A A│C C C T C│A│G G T C C C T G C C G A G G G A G T G G A T│
mScn10a cds (GRI) G│A G G│G│A G G A│G│A G│C C C T C│G│G G T C C C T G C C G A G G G A G T G G A T│


                               3250              3260              3270              3280
Y09108-cds        │G A C A C A A G C T C C T C C G A G G G C A G C A C G G T G G A C T G C C C G G│
mScn10a cds (GRI) │G A C A C A A G C T C C T C C G A G G G C A G C A C G G T G G A C T G C C C G G│


                               3290              3300              3310              3320
Y09108-cds        │A C C C A G A G G A G A T C C T G A G G A A G A T C C C T G A G C T G G C G G A│
mScn10a cds (GRI) │A C C C A G A G G A G A T C C T G A G G A A G A T C C C T G A G C T G G C G G A│


                               3330              3340              3350              3360
Y09108-cds        │G G A│T│C T G G A C G A G C C C G A T G A C T G T T T C│A│C A G A A G G C T G C│
mScn10a cds (GRI) │G G A│G│C T G G A C G A G C C C G A T G A C T G T T T C│C│C A G A A G G C T G C│


                               3370              3380              3390              3400
Y09108-cds        │A C T C G C C G C T G T C C C T G C T G C A A A G T G A A C A C C A G T A A G T│
mScn10a cds (GRI) │A C T C G C C G C T G T C C C T G C T G C A A A G T G A A C A C C A G T A A G T│


                               3410              3420              3430              3440
Y09108-cds        C│T C C T T G G G C C A C│A│G G C T G G C A G G T G C G C A A A A C C T G T T A│
mScn10a cds (GRI) T│T C C T T G G G C C A C│G│G G C T G G C A G G T G C G C A A A A C C T G T T A│


                               3450              3460              3470              3480
Y09108-cds        │C C G C A T T G T G G A G C A C A G C T G G T T T G A G A G T T T│T│A T│C│A T C│
mScn10a cds (GRI) │C C G C A T T G T G G A G C A C A G C T G G T T T G A G A G T T T│C│A T│T│A T C│


                               3490              3500              3510              3520
Y09108-cds        │T T C A T G A T C C T G C T C A G C A G T G G A│A│C G C T G G C C T T T G A G G│
mScn10a cds (GRI) │T T C A T G A T C C T G C T C A G C A G T G G A│G│C G C T G G C C T T T G A G G│


                               3530              3540              3550              3560
Y09108-cds        │A T A A C T A C C T G G A A G A G│A A│A│C C C C G A G T G A A G T C T G T G C T│
mScn10a cds (GRI) │A T A A C T A C C T G G A A G A│A A A│G│C C C C G A G T G A A G T C T G T G C T│
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                              3610              3620              3630              3640
Y09108-cds      G A G A T G T T G C T C A A G T G G G T A G C C T A T G G C T T C A A A A A A T
mScn10a cds (GRI) G A G A T G T T G C T C A A G T G G G T A G C T T A T G G C T T C A A A A A A T


                              3650              3660              3670              3680
Y09108-cds      A T T T C A C C A A T G C C T G G T G C T G G C T G G A C T T C C T C A T C G T
mScn10a cds (GRI) A T T T C A C C A A T G C C T G G T G C T G G C T G G A C T T C C T C A T C G T


                              3690              3700              3710              3720
Y09108-cds      G A A C A T C T C C C T C A C A A G C C T C A T A G C C A A G A T C C T C G A G
mScn10a cds (GRI) G A A T A T C T C C C T C A C A A G C C T C A T A G C C A A G A T C C T C G A G


                              3730              3740              3750              3760
Y09108-cds      T A T T C A G A C G T G G C G T C C A T C A A A G C C C T T C G G A C T C T C C
mScn10a cds (GRI) T A T T C A G A C G T G G C G T C C A T C A A A G C C C T T C G G A C T C T C C


                              3770              3780              3790              3800
Y09108-cds      G T G C C C T C C G G C C G C T G C G G G C T C T G T C T C G A T T C G A A G G
mScn10a cds (GRI) G T G C C C T C C G G C C G C T G C G G G C T C T G T C T C G A T T C G A A G G


                              3810              3820              3830              3840
Y09108-cds      C A T G A G G G T A G T G G T G G A T G C C T T G G T G G G C G C C A T C C C C
mScn10a cds (GRI) C A T G A G G G T A G T G G T G G A T G C C T T G G T G G G C G C C A T T C C C


                              3850              3860              3870              3880
Y09108-cds      T C C A T C A T G A A C G T C C T C C T C G T C T G C C T C A T C T T C T G G C
mScn10a cds (GRI) T C C A T C A T G A A C G T C C T C C T C G T C T G C C T C A T C T T C T G G C


                              3890              3900              3910              3920
Y09108-cds      T C A T C T T C A G C A T C A T G G G T G T G A A C C T C T T C G C C G G G A A
mScn10a cds (GRI) T C A T C T T C A G C A T C A T G G G T G T G A A C C T C T T C G C C G G G A A


                              3930              3940              3950              3960
Y09108-cds      A T T T T C G A G A T G T G T C G A C A C C A G A A G C A A C C C A T T T T C C
mScn10a cds (GRI) A T T T T C G A G A T G T G T C G A C A C C A G A A G C A A C C C A T T T T C C


                              3970              3980              3990              4000
Y09108-cds      G T C G T G A A T T C G A C A T T C G T G A A T A A C A A G T C T G A C T G T C
mScn10a cds (GRI) G T C G T G A A T T C G A C A T T C G T G A C T A A C A A G T C T G A C T G T T


                              4010              4020              4030              4040
Y09108-cds      A C A A T C A A A A C A A T A C C G G C C A C T T C T T C T G G G T T A A C G T
mScn10a cds (GRI) A C A A T C A A A A C A A T A C T G G C C A C T T C T T C T G G G T T A A C G T


                              4050              4060              4070              4080
Y09108-cds      C A A A G T C A A C T T C G A C A A C G T C G C T A T G G G C T A C C T C G C G
mScn10a cds (GRI) C A A A G T C A A C T T C G A C A A C G T T G C T A T G G G C T A C C T C G C G
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                        4130              4140              4150              4160
Y09108-cds       T G T A T G C A G C T G T C G A T T C T C G A G A T A T C A A C A G T C A G C C
mScn10a cds (GRI) T G T A T G C A G C T G T C G A T T C T C G A G A T A T C A A C A G T C A G C C


                        4170              4180              4190              4200
Y09108-cds       C A A T T G G G A G G A G A G C C T G T A C A T G T A C C T A T A C T T C G T C
mScn10a cds (GRI) C A A T T G G G A G G A G A G C C T G T A C A T G T A C C T A T A C T T C G T C


                        4210              4220              4230              4240
Y09108-cds       G T C T T C A T C A T T T T C G G T G G C T T C T T C A C G C T G A A T C T C T
mScn10a cds (GRI) G T C T T C A T C A T T T T C G G T G G C T T C T T C A C G C T G A A T C T C T


                        4250              4260              4270              4280
Y09108-cds       T T G T C G G G G T C A T C A T C G A C A A C T T C A A T C A A C A G A A A A A
mScn10a cds (GRI) T T G T C G G G G T C A T C A T T G A C A A C T T C A A T C A A C A G A A A A A


                        4290              4300              4310              4320
Y09108-cds       A A A G T T G G G G G G C C A G G A C A T C T T C A T G A C G G A A G A A C A A
mScn10a cds (GRI) A A A G C T A G G G G G C C A G G A C A T C T T C A T G A C A G A G G A G C A G


                        4330              4340              4350              4360
Y09108-cds       A A A A A A A T A T T A C A A T G C C A T G A A A A A A C T G G G C T C C A A A A
mScn10a cds (GRI) A A G A A G T A C T A C A A T G C C A T G A A G A A G C T G G G C T C C A A G A


                        4370              4380              4390              4400
Y09108-cds       A A C C C C A A A A G C C C A T C C C A C G G C C T C T G A A T A A G T A C C A
mScn10a cds (GRI) A A C C C C A G A A G C C C A T C C C A C G G C C T T T G A A T A A G T A C C A


                        4410              4420              4430              4440
Y09108-cds       G G G C T T C G T G T T T G A C A T T G T G A C C A G G C A A G C C T T T G A C
mScn10a cds (GRI) G G G C T T C G T G T T T G A C A T T G T G A C C A G G C A A G C A T T T G A C


                        4450              4460              4470              4480
Y09108-cds       A T C A T C A T C A T G G T T C T C A T C T G C C T C A A C A T G A T C A C C A
mScn10a cds (GRI) A T C A T C A T C A T G G C T C T C A T C T G C C T C A A C A T G A T C A C C A


                        4490              4500              4510              4520
Y09108-cds       T G A T G G T G G A G A C C G A C A A T C A G A G C G A G G A G A A G A C G A A
mScn10a cds (GRI) T G A T G G T G G A G A C C G A C A A T C A G A G C G A G G A G A A G A C G A A


                        4530              4540              4550              4560
Y09108-cds       G G T T C T G G G C A G A A T C A A C C A G T T T T T T G T G G C G G T C T T C
mScn10a cds (GRI) G G T C C T G G G C A G A A T C A A C C A G T T C T T C G T G G C C G T C T T C


                        4570              4580              4590              4600
Y09108-cds       A C G G G C G A G T G T G T G A T G A A A G T G T T C G C C C T G C G A C A G T
mScn10a cds (GRI) A C G G G C G A G T G T G T G A T G A A G A T G T T C G C C C T T C G G C A G T
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                        4650              4660              4670              4680
Y09108-cds       G G T G A T T C T G T C C A T T G C G A G T C T G T T G T T T T C T G C A A T C
mScn10a cds (GRI) G G T G A T T C T G T C C A T T T C T A G T C T G T T G T T T T C T G C G A T C
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                        4690              4700              4710              4720
Y09108-cds       C T T A A G T C A C T A G A A A G T T A C T T C T C C C C C A C G T T C T T C C
mScn10a cds (GRI) C T T A G C T C A C T A G A A A G T T A C T T C T C C C C C A C G C T C T T A C
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                        4730              4740              4750              4760
Y09108-cds       G C G T C A T C C G T C T G G C C A G G A T C G G C C G C A T C C T C A G G C T
mScn10a cds (GRI) G C G T C A T C C G T C T G G C C A G G A T C G G C C G C A T C C T C A G G C T
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                        4770              4780              4790              4800
Y09108-cds       G A T T C G A G C A G C C A A G G G G A T T C G C A C G C T G C T C T T C G C C
mScn10a cds (GRI) G A T T C G A G C A G C C A A G G G G A T T C G C A C G C T G C T C T T C G C C
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                        4810              4820              4830              4840
Y09108-cds       C T C A T G A T G T C C C T G C C C G C C C T C T T C A A C A T C G C T C T C C
mScn10a cds (GRI) C T C A T G A T G T C C C T G C C C G C C C T C T T C A A C A T C G G C C T C C
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                        4850              4860              4870              4880
Y09108-cds       T C C T C T T C C T C G T C A T G T T C A T C T A C T C C A T C T T C G G C A T
mScn10a cds (GRI) T C C T C T T C C T C G T C A T G T T C A T C T A C T C C A T C T T C G G C A T
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                        4890              4900              4910              4920
Y09108-cds       G G C C A G C T T C G C T A A T G T C A T A G A T G A G G C T G G C A T C G A C
mScn10a cds (GRI) G A C C A G C T T C G C T A A T G T C A T A G A T G A G G C T G G C A T C G A C
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                        4930              4940              4950              4960
Y09108-cds       G A C A T G T T C A A C T T C A A G A C C T T T G G C A A C A G C A T G C T G T
mScn10a cds (GRI) G A C A T G T T C A A C T T C A A G A C C T T T G G C A A C A G C A T G C T G T
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                        4970              4980              4990              5000
Y09108-cds       G C C T T T T C C A G A T C A C C A C G T C G G C T G G C T G G G A T G G C C T
mScn10a cds (GRI) G C C T T T T C C A G A T C A C C A C G T C G G C T G G C T G G G A T G G C C T
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                        5010              5020              5030              5040
Y09108-cds       C C T C A G C C C C A T C C T C A A C A C A G G A C C C C C C T A C T G C G A C
mScn10a cds (GRI) C C T C A G C C C C A T C C T C A A C A C A G G A C C C C C C T A C T G C G A C
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                        5050              5060              5070              5080
Y09108-cds       C C C A A C C G G C C C A A C A G C A A T G G C T C C A A G G G G A A T T G T G
mScn10a cds (GRI) C C C A A C C G G C C C A A C A G C A A T G G C T C C A A G G G G A A T T G T G
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                        5090              5100              5110              5120
Y09108-cds       G A A G C C C A G C G G T G G G C A T C C T C T T C T T C A C C A C C T A C A T
mScn10a cds (GRI) G A A G C C C A G C G G T G G G C A T C C T C T T C T T C A C C A C C T A C A T
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                              5170                5180                5190                5200
Y09108-cds        G T G A T T C T G G A G A A C T T C A A T G T G G C C A C A G A A G A G A G C A
mScn10a cds (GRI) G T G A T T C T G G A G A A C T T C A A T G T G G C C A C A G A A G A G A G C A


                              5210                5220                5230                5240
Y09108-cds        C G G A G C C C C T G A G C G A G G A C G A C T T T G A C A T G T T C T A T G A
mScn10a cds (GRI) C G G A G C C C C T G A G C G A G G A C G A C T T T G A C A T G T T C T A T G A


                              5250                5260                5270                5280
Y09108-cds        G A C C T G G G A G A A G T T T G A C C C G G A G G C C A C C C A G T T C A T T
mScn10a cds (GRI) G A C C T G G G A G A A G T T T G A C C C G G A G G C C A C C C A G T T C A T T


                              5290                5300                5310              5320
Y09108-cds        G C C T T T T C T G C C C T C T C A G A C T T T G C A G A C A C A C T C T C  C G
mScn10a cds (GRI) G C C T T T T C T G C C C T C T C A G A C T T T G C A G A C A C A C T C T C  T G


                              5330                5340                5350                5360
Y09108-cds        G C C C T C T T A G A A T C C C A A A A C C T A A T C A G A A T A T A T T A A T
mScn10a cds (GRI) G C C C T C T T A G A A T C C C A A A A C C T A A T C A G A A T A T A T T A A T


                              5370                5380                5390                5400
Y09108-cds        C C A G A T G G A C C T G C C G T T G G T C C C C G G A G A T A A G A T C C A C
mScn10a cds (GRI) C C A G A T G G A C C T G C C G T T G G T C C C C G G A G A T A A G A T C C A C


                              5410                5420                5430                5440
Y09108-cds        T G T T T G G A C A T C C T C T T T G C C T T C A C A A A G A A T G T C T T G G
mScn10a cds (GRI) T G T T T G G A C A T C C T C T T T G C C T T C A C A A A G A A T G T C T T G G


                              5450                5460                5470                5480
Y09108-cds        G A G A A T C T G G G G A G T T G G A T T C T C T G A A G A C T A A T A T G G A
mScn10a cds (GRI) G A G A A T C T G G G G A G T T G G A T T C T C T G A A G A C T A A T A T G G A


                              5490                5500                5510                5520
Y09108-cds        A G A G A A G T T T A T G G C A A C T A A T C T T T C C A A A G C A T C C T A T
mScn10a cds (GRI) A G A G A A G T T T A T G G C A A C T A A T C T T T C C A A A G C A T C C T A T


                              5530                5540                5550                5560
Y09108-cds        G A A C C A A T A G C A A C C A C C C T C C G G T G C A A G C A G G A A G A C A
mScn10a cds (GRI) G A A C C A A T A G C A A C C A C C C T C C G G T G C A A G C A G G A A G A C A


                              5570                5580                5590                5600
Y09108-cds        T C T C A G C C A C C A T T A T T C A A A A G G C C T A T C G G A A C T A C A T
mScn10a cds (GRI) T C T C A G C C A C C A T T A T T C A A A A G G C C T A T C G G A A C T A C A T


                              5610                5620                5630              5640
Y09108-cds        G T T G C A A C G C T C C T T G A T G C T C T C C A A C A C C C T G C A T G T G
mScn10a cds (GRI) G T T G C A A C G C T C C T T G A T G C T C T C C A A C C C C C T G C A T G T G
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                         5690               5700               5710               5720
Y09108-cds       G C T A T G T T A C A T T C A T G G C A A A T G A C A A C G G T G G G C T C C C
mScn10a cds (GRI)  G C T A T G T T A C A T T C A T G G C A A A T G A C A A C G G T G G G C T C C C


                         5730               5740               5750               5760
Y09108-cds       A G A C A A A T C A G A A A C T G C T T C T G C T A C G T C T T T C C C A C C A
mScn10a cds (GRI)  A G A C A A A T C G G A A A C T G C T T C T G C T A C G T C T T T C C C A C C A


                         5770               5780               5790               5800
Y09108-cds       T C C T A T G A A A G C G T C A C C A G G G G C C T G A G T G A C A G G G C C A
mScn10a cds (GRI)  T C C T A T G A C A G C G T C A C C A G G G G C C T G A G T G A C A G G G C C A


                         5810               5820               5830               5840
Y09108-cds       A C A T T A A C A C A T C T A G C T C A A T G C A A A A T G A A G A T G A A G T
mScn10a cds (GRI)  A C A T T A G C A C A T C T A G C T C A A T G C A A A A T G A A G A T G A A G T


                         5850               5860               5870               5880
Y09108-cds       C A C T G C T A A G G A A G G G A A T A G C C C T G G A C C T C A G T G A
mScn10a cds (GRI)  C A C T G C T A A G G A A G G G A A G A G C C C T G G A C C T C A G T G A
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Appendix C

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                        10              20              30            40
Y09108        M E F P F G S V G T T N F R R F T P E S L A E I E K Q I A A H R A A K K G R T K
mScn10a(GRI)  M E F P F G S V G T T N F R R F T P G S L A E I E K Q I A A H R A A K K G R P K


                        50              60              70            80
Y09108        Q R G Q K D K S E K P R P Q L D L K A C N Q L P R F Y G E L P A E L V G E P L E
mScn10a(GRI)  Q R G Q K D K S E K P R P Q L D L K A C N Q L P R F Y G E L P A E L V G E P L E


                        90              100             110           120
Y09108        D L D P F Y S T H R T F I L L N K S R T I S R F S A T W A L W L F S P F N L I R
mScn10a(GRI)  D L D P F Y S T H R T F I V L D K S R T I S R F S A T W A L W L F S P F N L I R


                        130             140             150           160
Y09108        R T A I K V S V H S W F S I F I T V T I L V N C V C M T R T D L P E K L E Y V F
mScn10a(GRI)  R T A I K V S V H S W F S I F I T V T I L V N C V C M T R T D L P E K L E Y A F


                        170             180             190           200
Y09108        T V V Y T F E A L I K I L A R G F C L N E F T Y L R D P W N W L D F S V I T L A
mScn10a(GRI)  T V V Y T F E A L I K I L A R G F C L N E F T Y L R D P W N W L D F S V I T L A


                        210             220             230           240
Y09108        Y V G A A V D L R G I S G L R T F R V L R A L K T V S V I P G L K V I V G A L I
mScn10a(GRI)  Y V G A A I D L R G I S G L R T F R V L R A L K T V S V I P G L K V I V G A L I


                        250             260             270           280
Y09108        H S V R K L A D V T I L T V F C L S V F A L V G L Q L F K G N L K N K C I K N G
mScn10a(GRI)  H S V R K L A D V T I L T V F C L S V F A L V G L Q L F K G N L K N K C I K N G


                        290             300             310           320
Y09108        T D P H K A D N L S S E M A E D I F I K P G T T D P L L C G N G S D A G H C P N
mScn10a(GRI)  T D P H K A D N L S S E M A G D I F I K P G T T D P L L C G N G S D A G H C P N


                        330             340             350           360
Y09108        G Y V C Q K T P D N P D F N Y T S F D S F A W A F L S L F R L M T Q D S W E R L
mScn10a(GRI)  D Y V C R K T S D N P D F N Y T S F D S F A W A F L S L F R L M T Q D S W E R L


                        370             380             390           400
Y09108        Y Q Q T L R A S G K M Y M V F F V L V I F L G S F Y L V N L I L A V V T M A Y E
mScn10a(GRI)  Y Q Q T L R A S G K M Y M V F F V L V I F L G S F Y L V N L I L A V V T M A Y E


                        410             420             430           440
Y09108        E Q S Q A T I A E I E A K E K K F Q E A L E V L Q K E Q E V L A A L G I D T T S
mScn10a(GRI)  E Q S Q A T I A E I E A K E K K F K E A L E V L Q K E Q E V L A A L G I G T T S
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                       490               500               510               520
Y09108       YNQRRMSFLGLSSGRRRASH G SVFHFRAPSQDVSFPDGIL
mScn10a(GRI)  YNQRRMSFLGLSSGRRRASH S SVFHFRAPSQDVSFPDGIL


                       530               540               550               560
Y09108       DDGVFHGDQESRR N SILLGRGAGQAGPLPRSPLPQSPNPG
mScn10a(GRI)  DDGVFHGDQESRR S SILLGRGAGQAGPLPRSPLPQSPNPG


                       570               580               590               600
Y09108       R KHGK EGQ L GM PTGELA A G T PEGPALDAAGQKNFLSA G YL
mScn10a(GRI)  P RRGE EGQ R GV PTGELA T GA  PEGPALDAAGQKNFLSA D YL


                       610               620               630               640
Y09108       NEPFRAQRAMSVVSIMTSVIEELEESKLKCPPCLIS F AQK
mScn10a(GRI)  NEPFRAQRAMSVVSIMTSVIEELEESKLKCPPCLIS L AQK


                       650               660               670               680
Y09108       YLIWECCPKWRKFKMVL L ELVTDPFAELTITLCIVVNTVF
mScn10a(GRI)  YLIWECCPKWKKFKMVL F ELVTDPFAELTITLCIVVNTVF


                       690               700               710               720
Y09108       MAMEHYPMTDAFDAMLQAGNIVFTVFFTMEMAFKIIAFDP
mScn10a(GRI)  MAMEHYPMTDAFDAMLQAGNIVFTVFFTMEMAFKIIAFDP


                       730               740               750               760
Y09108       YYYFQKKWNIFDCVIVTVSLLELS A SKKGSLSVLRS L RLL
mScn10a(GRI)  YYYFQKKWNIFDCVIVTVSLLELS T SKKGSLSVLRT F RLL


                       770               780               790               800
Y09108       RVFKLAKSWPTLNMLIKIIGNSVGALGNLTFILAIIVFIF
mScn10a(GRI)  RVFKLAKSWPTLNMLIKIIGNSVGALGNLTFILAIIVFIF


                       810               820               830               840
Y09108       ALVGKQLLSENYGCRRDGVSVWNGEKLRWHMCDFFHSFLV
mScn10a(GRI)  ALVGKQLLSENYGCRRDGISVWNGERLRWHMCDFFHSFLV


                       850               860               870               880
Y09108       VFRILCGEWIENMWVCMEVSQ N YICLTLFLTVMVLGNLVV
mScn10a(GRI)  VFRILCGEWIENMWVCMEVSQ D YICLTLFLTVMVLGNLVV


                       890               900               910               920
Y09108       LNLFIALLLNSFSADNLTAPEDDGEVNNLQLALARIQV L G
mScn10a(GRI)  LNLFIALLLNSFSADNLTAPEDDGEVNNLQVALARIQV F G


                       930               940               950               960
Y09108       HRASRAITSYIRSHCR F RWPKVETQLGMKPPLTSCKVENH
mScn10a(GRI)  HRASRAITSYIRSHCR L RWPKVETQLGMKPPLTSCKAENH
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                  1010              1020              1030              1040
Y09108      A E G E S D L D E L E E D V E Q A S Q S S W Q E E S P K G Q Q E L L P Q V Q K C
mScn10a(GRI) A E G E S D L D E L E E D V E H A S Q S S W Q E E S P K G Q - E L L Q Q V Q K C


                  1050              1060              1070              1080
Y09108      E N H Q A A R S P T S G M S S E D L A P Y L G E R W K R K D N P Q V P A E G V D
mScn10a(GRI) E D H Q A A R S P P S G M S S E D L A P Y L G E R W Q R E E S P R V P A E G V D


                  1090              1100              1110              1120
Y09108      D T S S S E G S T V D C P D P E E I L R K I P E L A E D L D E P D D C F T E G C
mScn10a(GRI) D T S S S E G S T V D C P D P E E I L R K I P E L A E E L D E P D D C F P E G C


                  1130              1140              1150              1160
Y09108      T R R C P C C K V N T S K S P W A T G W Q V R K T C Y R I V E H S W F E S F I I
mScn10a(GRI) T R R C P C C K V N T S K F P W A T G W Q V R K T C Y R I V E H S W F E S F I I


                  1170              1180              1190              1200
Y09108      F M I L L S S G T L A F E D N Y L E E K P R V K S V L E Y T D R V F T F I F V F
mScn10a(GRI) F M I L L S S G A L A F E D N Y L E E K P R V K S V L E Y T D R V F T F I F V F


                  1210              1220              1230              1240
Y09108      E M L L K W V A Y G F K K Y F T N A W C W L D F L I V N I S L T S L I A K I L E
mScn10a(GRI) E M L L K W V A Y G F K K Y F T N A W C W L D F L I V N I S L T S L I A K I L E


                  1250              1260              1270              1280
Y09108      Y S D V A S I K A L R T L R A L R P L R A L S R F E G M R V V V D A L V G A I P
mScn10a(GRI) Y S D V A S I K A L R T L R A L R P L R A L S R F E G M R V V V D A L V G A I P


                  1290              1300              1310              1320
Y09108      S I M N V L L V C L I F W L I F S I M G V N L F A G K F S R C V D T R S N P F S
mScn10a(GRI) S I M N V L L V C L I F W L I F S I M G V N L F A G K F S R C V D T R S N P F S


                  1330              1340              1350              1360
Y09108      V V N S T F V N N K S D C H N Q N N T G H F F W V N V K V N F D N V A M G Y L A
mScn10a(GRI) V V N S T F V T N K S D C Y N Q N N T G H F F W V N V K V N F D N V A M G Y L A


                  1370              1380              1390              1400
Y09108      L L Q V A T F K G W M D I M Y A A V D S R D I N S Q P N W E E S L Y M Y L Y F V
mScn10a(GRI) L L Q V A T F K G W M D I M Y A A V D S R D I N S Q P N W E E S L Y M Y L Y F V


                  1410              1420              1430              1440
Y09108      V F I I F G G F F T L N L F V G V I I D N F N Q Q K K K L G G Q D I F M T E E Q
mScn10a(GRI) V F I I F G G F F T L N L F V G V I I D N F N Q Q K K K L G G Q D I F M T E E Q


                  1450              1460              1470              1480
Y09108      K K Y Y N A M K K L G S K K P Q K P I P R P L N K Y Q G F V F D I V T R Q A F D
mScn10a(GRI) K K Y Y N A M K K L G S K K P Q K P I P R P L N K Y Q G F V F D I V T R Q A F D
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                    1530            1540            1550            1560
Y09108       T G E C V M K V F A L R Q Y Y F T N G W N V F D F I V V I L S I A S L L F S A I
mScn10a(GRI) T G E C V M K M F A L R Q Y Y F T N G W N V F D F I V V I L S I S S L L F S A I


                    1570            1580            1590            1600
Y09108       L K S L E S Y F S P T F F R V I R L A R I G R I L R L I R A A K G I R T L L F A
mScn10a(GRI) L S S L E S Y F S P T L L R V I R L A R I G R I L R L I R A A K G I R T L L F A


                    1610            1620            1630            1640
Y09108       L M M S L P A L F N I A L L L F L V M F I Y S I F G M A S F A N V I D E A G I D
mScn10a(GRI) L M M S L P A L F N I G L L L F L V M F I Y S I F G M T S F A N V I D E A G I D


                    1650            1660            1670            1680
Y09108       D M F N F K T F G N S M L C L F Q I T T S A G W D G L L S P I L N T G P P Y C D
mScn10a(GRI) D M F N F K T F G N S M L C L F Q I T T S A G W D G L L S P I L N T G P P Y C D


                    1690            1700            1710            1720
Y09108       P N R P N S N G S K G N C G S P A V G I L F F T T Y I I I S F L I V V N M Y I A
mScn10a(GRI) P N R P N S N G S K G N C G S P A V G I L F F T T Y I I I S F L I V V N M Y I A


                    1730            1740            1750            1760
Y09108       V I L E N F N V A T E E S T E P L S E D D F D M F Y E T W E K F D P E A T Q F I
mScn10a(GRI) V I L E N F N V A T E E S T E P L S E D D F D M F Y E T W E K F D P E A T Q F I


                    1770            1780            1790            1800
Y09108       A F S A L S D F A D T L S G P L R I P K P N Q N I L I Q M D L P L V P G D K I H
mScn10a(GRI) A F S A L S D F A D T L S G P L R I P K P N Q N I L I Q M D L P L V P G D K I H


                    1810            1820            1830            1840
Y09108       C L D I L F A F T K N V L G E S G E L D S L K T N M E E K F M A T N L S K A S Y
mScn10a(GRI) C L D I L F A F T K N V L G E S G E L D S L K T N M E E K F M A T N L S K A S Y


                    1850            1860            1870            1880
Y09108       E P I A T T L R C K Q E D I S A T I I Q K A Y R N Y M L Q R S L M L S N T L H V
mScn10a(GRI) E P I A T T L R C K Q E D I S A T I I Q K A Y R N Y M L Q R S L M L S N P L H V


                    1890            1900            1910            1920
Y09108       P R A E E D G V S L P K G G Y V T F M A N D N G G L P D K S E T A S A T S F P P
mScn10a(GRI) P R A E E D G V S L P R E G Y V T F M A N D N G G L P D K S E T A S A T S F P P


                    1930            1940            1950            1960
Y09108       S Y E S V T R G L S D R A N I N T S S S M Q N E D E V T A K E G N S P G P Q N
mScn10a(GRI) S Y D S V T R G L S D R A N I S T S S S M Q N E D E V T A K E G K S P G P Q N
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Appendix D

Activation properties of Scn10a channels heterologously expressed in sympathetic neurons

Cell mV	1	2	3	4	5	6	7	8	9	Mean	SEM	N
					Normalized Conductance							
-39	-0.003	-0.005	-0.001	-0.001	-0.003	-0.001	-0.003	-0.004	-0.004	-0.003	0.000	9
-34	-0.004	-0.006	-0.001	-0.003	-0.003	-0.001	-0.004	-0.004	-0.004	-0.003	0.001	9
-29	-0.005	-0.006	-0.002	-0.001	-0.004	0.000	-0.005	-0.005	-0.005	-0.003	0.001	9
-24	-0.003	-0.008	-0.002	-0.002	-0.001	0.002	-0.005	-0.003	-0.004	-0.003	0.001	9
-19	0.000	-0.004	0.000	0.000	0.003	0.006	-0.003	-0.002	-0.003	0.000	0.001	9
-14	0.011	0.006	0.006	0.011	0.015	0.025	0.005	0.005	0.003	0.010	0.002	9
-9	0.049	0.035	0.021	0.026	0.047	0.086	0.023	0.016	0.014	0.035	0.008	9
-4	0.153	0.122	0.068	0.064	0.111	0.203	0.091	0.044	0.051	0.101	0.018	9
1	0.343	0.294	0.179	0.152	0.236	0.353	0.235	0.125	0.154	0.230	0.028	9
6	0.554	0.529	0.346	0.280	0.396	0.501	0.430	0.268	0.322	0.403	0.036	9
11	0.722	0.750	0.508	0.431	0.558	0.625	0.624	0.439	0.502	0.573	0.038	9
16	0.872	0.905	0.661	0.575	0.718	0.736	0.775	0.599	0.664	0.723	0.038	9
21	0.963	1.001	0.782	0.727	0.847	0.822	0.896	0.752	0.793	0.843	0.031	9
26	1.011	0.958	0.890	0.837	0.919	0.909	0.965	0.868	0.900	0.917	0.018	9
31	0.966	0.753	0.978	0.934	0.972	1.004	0.993	0.957	0.984	0.949	0.025	9

Boltzmann equation parameters as determined by nonlinear regression

	1	2	3	4	5	6	7	8	9	Mean	SEM	N
V_h	5.07	5.42	11.21	13.49	9.33	6.90	8.12	13.16	11.43	9.35	1.06	9
k	5.43	4.75	6.73	7.26	6.72	7.81	5.74	6.53	6.30	6.36	0.31	9

Inactivation properties of Scn10a channels heterologously expressed in sympathetic neurons

Cell mV	1	2	3	4	Mean	SEM	N
		Normalized conductance					
-59	1.000	1.000	1.000	1.000	1.000	0.000	4
-53	1.011	0.998	1.001	0.985	0.999	0.005	4
-48	0.960	0.992	0.952	0.976	0.970	0.009	4
-44	0.956	0.963	0.883	0.933	0.934	0.018	4
-38	0.936	0.918	0.772	0.840	0.866	0.038	4
-34	0.907	0.841	0.611	0.717	0.769	0.066	4
-28	0.825	0.723	0.432	0.539	0.630	0.089	4
-23	0.658	0.560	0.278	0.348	0.461	0.089	4
-18	0.388	0.347	0.145	0.147	0.257	0.065	4
-14	0.127	0.137	0.046	0.046	0.089	0.025	4
-9	0.027	0.034	0.017	0.023	0.025	0.004	4
-4	0.004	0.008	0.004	0.009	0.006	0.001	4
1	0.000	0.000	0.000	0.000	0.000	0.000	4

Boltzmann equation parameters as determined by nonlinear regression

	1	2	3	4	Mean	SEM	N
V_h	-20.68	-22.51	-30.14	-27.66	-25.25	2.20	4
K	-4.30	-5.56	-6.52	-5.89	-5.57	0.47	4

Appendix E

Activation properties of TTX-R sodium channels in mouse DRG neurons

Cell mV	1	2	3	4	5	6	7	8	9	Mean	SEM	N
					Normalized Conductance							
-58	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.0007	0.0001	9
-48	0.000	-0.002	0.000	0.000	0.001	0.000	0.000	-0.001	0.000	-0.0001	0.0003	9
-43	0.001	-0.001	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.0003	0.0002	9
-38	0.001	-0.001	0.000	0.002	0.004	-0.001	0.000	0.002	-0.001	0.0007	0.0005	9
-34	0.003	0.002	0.002	0.004	0.007	0.001	0.001	0.005	0.000	0.0028	0.0007	9
-28	0.006	0.007	0.006	0.009	0.010	0.009	0.005	0.009	0.002	0.0069	0.0008	9
-24	0.013	0.022	0.016	0.020	0.017	0.029	0.015	0.018	0.007	0.0175	0.0021	9
-19	0.032	0.057	0.046	0.047	0.032	0.082	0.048	0.042	0.022	0.0454	0.0058	9
-14	0.086	0.161	0.127	0.110	0.060	0.223	0.162	0.105	0.062	0.1217	0.0177	9
-9	0.244	0.373	0.312	0.268	0.134	0.437	0.433	0.271	0.184	0.2950	0.0349	9
-4	0.511	0.625	0.558	0.493	0.300	0.663	0.716	0.531	0.405	0.5335	0.0429	9
1	0.716	0.796	0.746	0.688	0.519	0.839	0.888	0.757	0.633	0.7313	0.0369	9
6	0.852	0.903	0.861	0.824	0.718	0.947	0.978	0.914	0.804	0.8668	0.0265	9
11	0.935	0.969	0.933	0.921	0.859	0.999	1.000	0.988	0.923	0.9475	0.0153	9
16	0.982	1.000	0.988	0.983	0.957	1.000	0.963	1.000	0.986	0.9843	0.0053	9
21	1.000	0.985	1.000	1.000	1.000	0.903	0.852	0.924	1.000	0.9628	0.0185	9

Boltzmann equation parameters as determined by nonlinear regression

	1	2	3	4	5	6	7	8	9	Mean	SEM	N
Vh	-3.61	-6.20	-4.72	-3.38	0.72	-7.56	-7.73	-4.48	-1.69	-4.29	0.91	9
k	4.94	4.97	5.25	5.53	5.40	5.00	3.99	4.55	4.99	4.96	0.15	9

Inactivation properties of TTX-R sodium channels in mouse DRG neurons

Cell mV	1	2	3	4	5	6	7	8	9	Mean	SEM	N
					Normalized Conductance							
-58	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.0000	0.0000	9
-53	0.972	0.990	0.988	1.009	1.002	0.961	0.989	0.991	0.988	0.9877	0.0047	9
-48	0.919	0.963	0.944	0.996	1.013	0.913	0.952	0.965	0.950	0.9572	0.0108	9
-43	0.810	0.878	0.869	0.943	0.974	0.805	0.870	0.892	0.859	0.8777	0.0183	9
-38	0.625	0.759	0.732	0.815	0.875	0.620	0.697	0.726	0.663	0.7236	0.0282	9
-33	0.369	0.567	0.513	0.583	0.680	0.420	0.421	0.452	0.390	0.4885	0.0347	9
-29	0.140	0.321	0.266	0.272	0.377	0.222	0.158	0.184	0.148	0.2321	0.0277	9
-23	0.046	0.125	0.087	0.079	0.145	0.125	0.049	0.084	0.058	0.0888	0.0119	9
-19	0.023	0.035	0.032	0.033	0.050	0.078	0.023	0.060	0.035	0.0410	0.0061	9
-14	0.013	0.011	0.015	0.015	0.023	0.042	0.013	0.035	0.020	0.0210	0.0036	9
-9	0.003	0.003	0.006	0.005	0.010	0.021	0.005	0.021	0.007	0.0091	0.0023	9
-4	0.001	0.002	0.001	0.000	0.000	0.005	0.002	0.009	0.003	0.0025	0.0010	9
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.0000	9

Boltzmann equation parameters as determined by nonlinear regression

	1	2	3	4	5	6	7	8	9	Mean	SEM	N
Vh	-36.20	-32.35	-33.04	-32.68	-30.89	-35.43	-35.16	-34.53	-35.62	-33.99	0.60	9
k	-4.71	-4.78	-4.41	-3.66	-3.83	-5.54	-3.82	-3.97	-3.95	-4.30	0.21	9

```
                              20              40              60
          ATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
          ─────────────────────────────────────────────────────────────
9  4/25whole ▶   ATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
A  4/25whole ▶   ATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
B  4/25whole ▶   ATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀
```

```
                              80              100             120
          TYCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTC
          ─────────────────────────────────────────────────────────────
9  4/25whole ▶   TTCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTC
A  4/25whole ▶   TCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTC
B  4/25whole ▶   TCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTC
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀
```

```
                              140             160             180
          TCCTTCTCCYTCTCCTTCTNN..INNN..IN..NNN.NI.A.NNNNNNNNNNNNNNNNNNNN
          ─────────────────────────────────────────────────────────────
9  4/25whole ▶   TCCTTCTCCTTCTCCTTCTCCTTCTCCTTCCCCTTCTCCTTCTCCTTCTCCTTCTCCTTC
A  4/25whole ▶   TCCTTCTCCTTCTCCTTCT..............................................
B  4/25whole ▶   TCCTTCTCCCTCTCCTTCTCCTTCT........................................
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀
```

```
                              200              220              240
     █CCCTTTTCTCTGCCTCCTCCTCCTTCTCC█CCTCCTTCTTCTCCTTCCTCTTCTTCCTT
```

9 4/25whole ▶ TCCCTTTTCTCTGCCTCCTCCTCCTTCTCCTCCTCCTTCTTCTCCTTCCTCTTCTTCCTT

A 4/25whole ▶ .CCCTTTTCTCTGCCTCCTCCTCCTTCTCCACCTCCTTCTTCTCCTTCCTCTTCTTCCTT

B 4/25whole ▶ .CCCTTTTCTCTGCCTCCTCCTCCTTCTCCTCCTCCTTCTTCTCCTTCCTCTTCTTCCTT

Intron1-in/A-NotI ▶

Intron1-in/B-NotI ▶

Intron1-out/A-SwaI ◀

Intron1-out/B-SwaI ◀

Intron1-out/A-SwaI/I-CeuI ◀

Intron1-out/B-SwaI/I-CeuI ◀

```
                              260              280              300
     CTCTTCTTCT█NWYYK█CRKYSBY█CCTTTCGAATGAC█AT█SKM█GSMMRY█GACAACCAACAAT
```

9 4/25whole ▶ CTCTTCTTCTTTCTTCATTGCTCCTTTCGAATGACTATGTAGCAAACGACAACCAACAAT

A 4/25whole ▶ CTCTTCTTCTTTCTTCATTGTTCCTTTCGAATGACTATGTAGCAAATGACAACCAACAAT

B 4/25whole ▶ CTCTTCTTCTTTCTTCATTGTTCCTTTCGAATGACTATGTAGCAAATGACAACCAACAAT

Intron1-in/A-NotI ▶   GATCGCGGCCGCCCTTTCGAATGACTATGTAGC

Intron1-in/B-NotI ▶   GATCGCGGCCGCGACAACCAACAAT

Intron1-out/A-SwaI ◀

Intron1-out/B-SwaI ◀

Intron1-out/A-SwaI/I-CeuI ◀

Intron1-out/B-SwaI/I-CeuI ◀

```
                              320              340              360
     CAGCAACCTACCCATAGGGGCTCTAGAATTTATGTCCTCTGAGAG█RY█CCCAGAATTCCAA
```

9 4/25whole ▶ CAGCAACCTACCCATAGGGGCTCTAGAATTTATGTCCTCTGAGAGACCCCAGAATTCCAA

A 4/25whole ▶ CAGCAACCTACCCATAGGGGCTCTAGAATTTATGTCCTCTGAGAGTTCCCAGAATTCCAA

B 4/25whole ▶ CAGCAACCTACCCATAGGGGCTCTAGAATTTATGTCCTCTGAGAGTCCCCAGAATTCCAA

Intron1-in/A-NotI ▶

Intron1-in/B-NotI ▶ CAGCAACC

Intron1-out/A-SwaI ◀

Intron1-out/B-SwaI ◀

Intron1-out/A-SwaI/I-CeuI ◀

Intron1-out/B-SwaI/I-CeuI ◀

```
                                    380              400              420
        ATGTCACNCACTTGCAGAAACTACCTGCAACCGACAAANTCTCGCCCCTGCTAGAGCAAG
```

9　4/25whole ▶　ATGTCACACACTTGCAGAAACTACCTGCAACCGACAAAGTCTCGCCCCTGCTAGAGCAAG

A　4/25whole ▶　ATGTCACACACTTGCAGAAACTACCTGCAACCGACAAAATCTCGCCCCTGCTAGAGCAAG

B　4/25whole ▶　ATGTCACGCACTTGCAGAAACTACCTGCAACCGACAAAATCTCGCCCCTGCTAGAGCAAG

Intron1-in/A-NotI ▶

Intron1-in/B-NotI ▶

Intron1-out/A-SwaI ◀

Intron1-out/B-SwaI ◀

Intron1-out/A-SwaI/I-CeuI ◀

Intron1-out/B-SwaI/I-CeuI ◀

```
                                    440              460              480
        AGGCAAATCATAGTCAGCTGCTGTGGNTCAATCYKNAAGCAGCTCCATATCCCACACCCG
```

9　4/25whole ▶　AGGCAAATCATAGTCAGCTGCTGTGG.TCAATCCG.AAGCAGCTCCATATCCCACACCCG

A　4/25whole ▶　AGGCAAATCATAGTCAGCTGCTGCGGGTCAATCTTGAAGCAGCTCCATATCCCACACCCG

B　4/25whole ▶　AGGCAAATCATAGTCAGCTGCTGTGG.TCAATCTG.AAGCAGCTCCATATCCCACACCCG

Intron1-in/A-NotI ▶

Intron1-in/B-NotI ▶

Intron1-out/A-SwaI ◀

Intron1-out/B-SwaI ◀

Intron1-out/A-SwaI/I-CeuI ◀

Intron1-out/B-SwaI/I-CeuI ◀

```
                                    500              520              540
        GGATTAAAACAGACATACTCATAATATTNCTGNGATTYTCTTCTTAAAGAAANGCAAAAT
```

9　4/25whole ▶　GGATTAAAACAGACATACTCATAATATTTCTGTGATTTTCTTCTTAAAGAAA.GCAAAAT

A　4/25whole ▶　GGATTAAAACAGACATACTCATAATATTTCTGGGATTCTCTTCTTAAAGAAAAGCAAAAT

B　4/25whole ▶　GGATTAAAACAGACATACTCATAATATT.CTGTGATTCTCTTCTTAAAGAAA.GCAAAAT

Intron1-in/A-NotI ▶

Intron1-in/B-NotI ▶

Intron1-out/A-SwaI ◀

Intron1-out/B-SwaI ◀

Intron1-out/A-SwaI/I-CeuI ◀

Intron1-out/B-SwaI/I-CeuI ◀

```
                                        560              580              600
              TTNYACTGCAATGAGGGAAAGATGBTCARAATTTATAAAGCBTAGTTTGTGGGGGAATGG
              ------------------------------------------------------------
9  4/25whole▶ TTTCACTGCAATGAGGGAAAGATG.TCAAAATTTATAAAGC.TAGTTTGTGGGGGAATGG
A  4/25whole▶ TT.CACTGCAATGAGGGAAAGATG.TCAGAATTTATAAAGC.TAGTTTGTGGGGGAATGG
B  4/25whole▶ TTTTACTGCAATGAGGGAAAGATGGTCAAAATTTATAAAGCCTAGTTTGTGGGGGAATGG
Intron1-in/A-NotI▶
Intron1-in/B-NotI▶
Intron1-out/A-SwaI◀
Intron1-out/B-SwaI◀
Intron1-out/A-SwaI/I-CeuI◀
Intron1-out/B-SwaI/I-CeuI◀
```

```
                                        620              640              660
              GATGGAGCTTCTTACAAAGCAAGGAGNAARRCAATGGCTTCAGGGATATGGANCAAARAC
              ------------------------------------------------------------
9  4/25whole▶ GATGGAGCTTCTTACAAAGCAAGGAGGAAA.CAATGGCTTCAGGGATATGGAGCAAAGAC
A  4/25whole▶ GATGGAGCTTCTTACAAAGCAAGGAG.AAAGCAATGGCTTCAGGGATATGGAGCAAAGAC
B  4/25whole▶ GATGGAGCTTCTTACAAAGCAAGGAGGAAG.CAATGGCTTCAGGGATATGGAACAAAAAC
Intron1-in/A-NotI▶
Intron1-in/B-NotI▶
Intron1-out/A-SwaI◀
Intron1-out/B-SwaI◀
Intron1-out/A-SwaI/I-CeuI◀
Intron1-out/B-SwaI/I-CeuI◀
```

```
                                        680              700              720
              ACACCTGTGCTACCTGGATTTGTAGATGGACTGCAGAGGATGGAGGGGGGGGGGGNNNCGGG
              ------------------------------------------------------------
9  4/25whole▶ ACACCTGTGCTACCTGGATTTGTAGATGGACTGCAGAGGATGGAGGGGGGGGGGGG.CGGG
A  4/25whole▶ ACACCTGTGCTACCTGGATTTGTAGATGGACTGCAGAGGATGGAGGGGGGGGGGGGGCGGG
B  4/25whole▶ ACACCTGTGCTACCTGGATTTGTAGATGGACTGCAGAGGATGGAGGGGGGGGGG...CGGG
Intron1-in/A-NotI▶
Intron1-in/B-NotI▶
Intron1-out/A-SwaI◀
Intron1-out/B-SwaI◀
Intron1-out/A-SwaI/I-CeuI◀
Intron1-out/B-SwaI/I-CeuI◀
```

```
                                          740           760           780
           ▓TATTGA▓GAGCTGGAGACAGAGATAGGGGGCTGCTCTGTCAATCAAGTAGGGGTGATGG

9  4/25whole ▶    .TATTGAAGAGCTGGAGACAGAGATAGGGGGCTGCTCTGTCAATCAAGTAGGGGTGATGG
A  4/25whole ▶    .TATTGAGGAGCTGGAGACAGAGATAGGGGGCTGCTCTGTCAATCAAGTAGGGGTGATGG
B  4/25whole ▶    GTATTGAGGAGCTGGAGACAGAGATAGGGGGCTGCTCTGTCAATCAAGTAGGGGTGATGG
Intron1-in/A-Notl ▶
Intron1-in/B-Notl ▶
Intron1-out/A-Swal ◀
Intron1-out/B-Swal ◀
Intron1-out/A-Swal/I-Ceul ◀
Intron1-out/B-Swal/I-Ceul ◀
```

```
                                      800           820           840
           ACAGGAGGGTTGCAGTGGTCAGGCTGTCACCACTCTCTGG▓T▓▓YT▓CCT▓YMSR▓▓G▓K▓

9  4/25whole ▶    ACAGGAGGGTTGCAGTGGTCAGGCTGTCACCACTCTCTGGTTATTTTCCTCCCAGAAGAG
A  4/25whole ▶    ACAGGAGGGTTGCAGTGGTCAGGCTGTCACCACTCTCTGGTTATTTTCCTCCCAGAAGAG
B  4/25whole ▶    ACAGGAGGGTTGCAGTGGTCAGGCTGTCACCACTCTCTGGTTATTTTCCTCCCAGAAGAG
Intron1-in/A-Notl ▶
Intron1-in/B-Notl ▶
Intron1-out/A-Swal ◀                                                  GAG
Intron1-out/B-Swal ◀      GGTCAGGCTGTCACCACTCTCTGGATTT
Intron1-out/A-Swal/I-Ceul ◀                                          GAG
Intron1-out/B-Swal/I-Ceul ◀    GGTCAGGCTGTCACCACTCTCTGGTTCGCTACCTTAGGACCGTT
```

```
                                      860           880           900
           ▓KWRWW▓CSW▓TYYCCAAGAAG▓WYKMKA▓SWTRGRRYYSYY▓T▓KKKRYSMK▓T▓GGAACT

9  4/25whole ▶    TGTAAATCCTTCCCCAAGAAGAATGAGAAGATGGAGTTCCCCTTTGGGTCCGTGGGAACT
A  4/25whole ▶    TGTAAATCCTTCCCCAAGAAGAATGAGAAGATGGAGTTCCCCTTTGGGTCCGTGGGAACT
B  4/25whole ▶    TGTAAATCCTTCCCCAAGAAGAATGAGAAGATGGAGTTCCCCTTTGGGTCCGTGGGAACT
Intron1-in/A-Notl ▶
Intron1-in/B-Notl ▶
Intron1-out/A-Swal ◀      TGTAAATCCTTCCCCAAGAAGATTT
Intron1-out/B-Swal ◀
Intron1-out/A-Swal/I-Ceul ◀  TGTAAATCCTTCCCCAAGAAGTTCGCTACCTTAGGACCGTTATAGTTACGATTT
Intron1-out/B-Swal/I-Ceul ◀  ATAGTTACGATTT
```

```
                                        920              940            960
                            ACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGC
                           ┌──────┴───────┴───────┴───────┴──────┴───
9  4/25whole ▶              ACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGC
A  4/25whole ▶              ACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGC
B  4/25whole ▶              ACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGC
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀
```

Appendix G:

Primers for 5' Rapid Amplification of cDNA Ends:

GCCAGCGACTCTGGAGTGAACCGTC- Scn10a5'RACE1
GACCAGCTCTGCTGGGAGCTCGC- Scn10a5'RACE2
GAACCTGGGCAGCTGGTTACAGGCC- Scn10a5'RACE3

RACE products

Scn10a RACE Clone B from ATG out

Sequence Range: 1 to 245 5'UTR (245+ cds)

```
            10        20        30        40        50        60
        CCGGACAAGTGTAAGTTTCGCAGAGCTGGGGTCTCCAGCTTACTTCTGCTAATGCTACCC
        _____5' UTR MOUSE SCN10A_____>

            70        80        90        100       110       120
        CAGGCCTTTAGACGGAGAACAGATGGCAGATGGAGTTTCTTCCTGCCATGCGCGAATGCT
        _____5' UTR MOUSE SCN10A_____>

            130       140       150       160       170       180
        GAGCGGATCTCATGATCCCCGAGCTCATGGCTTTCAGTAGAGGCAACCTGGGCTAAGAAG
        _____5' UTR MOUSE SCN10A_____>

            190       200       210       220       230       240
        AGATCTCCGACTTACGGAGCAGCAAAGAAGAGTGTAAATCCTTCCCCAAGAAGAATGAGA
        _____5' UTR MOUSE SCN10A_____>


        >Start_codon
             |
             |
          AGATG
    GAGTTCCCCTTTGGGTCCGTGGGAACTACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGCAGAGATCGAGA
    AGCAGATCGNTGCCCACCGCGCCGCCAAGAAGGGCAGACCTAAGCAAAGAGGACAGAAGGACAAGAGTGAGAA
    GCCCAGGCCTCAGTTGGACTTGAAGGCCTGTAACCAGCTGCCCAGGTTC



Scn10a RACE Clone I from ATG out

Sequence Range: 1 to 209 5'UTR (210+ cds)

            10        20        30        40        50        60
        ACGCGGGGACTTCTGCTAATGCTACCCCAGGCCTTTAGACGGAGAACAGATGGCAGATGG
        _____SCN10A 5'UTR_____>

            70        80        90        100       110       120
        AGTTTCTTCCTGCCATGCGCGAATGCTGAGCGGGTCTCATGATCCCCGAGCTCATGGCTT
        _____SCN10A 5'UTR_____>

                                                  >splice_5'_end_of_exon
                                                              |
            130       140       150       160       170 |     180
        TCAGTAGAGGCAACCTGGGCTAAGAAGAGATCTCCGACTTACGGAGCAGCAAAGAGTGTA
        _____SCN10A 5'UTR_____>

                          >Start_codon
                               |
```

```
            190        200              |
    AATCCTTCCCCAAGAAGAATGAGAAGATG
    ____SCN10A 5'UT____>
```

```
GAGTTCCCCTTTGGGTCCGTGGGAACTACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGCAGAGATCGAGAAGCAGA
CCGCTGCCCACCGCGCCGCCAAGAAGGGCAGACCTAAGCAAAGAGGACAGAAGGACAAGAGTGAGAAGCCCAGGCCTCA
GTTGGACTTGAAGGCCTGTAACCAGCTGCCCAGGTTC
```

Appendix H:

LM-PCR primers: used for LM-PCR out (upstream) walking into the first intron 5' from the translational start site: (simply used RACE primers on genomic template instead of cDNA)

GCCAGCGACTCTGGAGTGAACCGTC- Scn10a5'RACE1
GACCAGCTCTGCTGGGAGCTCGC- Scn10a5'RACE2
GAACCTGGGCAGCTGGTTACAGGCC- Scn10a5'RACE3

Sequence of LM-PCR product generated by walking into the first intron 5' from the translational start site:

```
          10         20         30         40         50         60
ATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC

          70         80         90        100        110        120
TTCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTC

         130        140        150        160        170        180
TCCTTCTCCTTCTCCTTCTCCTTCTCCTTCCCCTTCTCCTTCTCCTTCTCCTTCTCCTTC

         190        200        210        220        230        240
TCCCTTTTCTCTGCCTCCTCCTCCTTCTCCTCCTCCTTCTTCTCCTTCCTCTTCTTCCTT

         250        260        270        280        290        300
CTCTTCTTCTTTCTTCATTGCTCCTTTCGAATGACTATGTAGCAAACGACAACCAACAAT

         310        320        330        340        350        360
CAGCAACCTACCCATAGGGGCTCTAGAATTTATGTCCTCTGAGAGACCCCAGAATTCCAA

         370        380        390        400        410        420
ATGTCACACACTTGCAGAAACTACCTGCAACCGACAAAGTCTCGCCCCTGCTAGAGCAAG

         430        440        450        460        470        480
AGGCAAATCATAGTCAGCTGCTGTGGTCAATCCGAAGCAGCTCCATATCCCACACCCGGG

         490        500        510        520        530        540
ATTAAAACAGACATACTCATAATATTTCTGTGATTTTCTTCTTAAAGAAAGCAAAATTTT

         550        560        570        580        590        600
CACTGCAATGAGGGAAAGATGTCAAAATTTATAAAGCTAGTTTGTGGGGGAATGGGATGG

         610        620        630        640        650        660
AGCTTCTTACAAAGCAAGGAGGAAACAATGGCTTCAGGGATATGGAGCAAAGACACACCT

         670        680        690        700        710        720
GTGCTACCTGGATTTGTAGATGGACTGCAGAGGATGGAGGGGGGGGGGGCGGGTATTGAA

         730        740        750        760        770        780
GAGCTGGAGACAGAGATAGGGGGCTGCTCTGTCAATCAAGTAGGGGTGATGGACAGGAGG

         790        800        810        820        830        840
GTTGCAGTGGTCAGGCTGTCACCACTCTCTGGTTATTTTCCTCCCAGAAGAGTGTAAATC

         850        860        870        880        890        900
CTTCCCCAAGAAGAATGAGAAGATGGAGTTCCCCTTTGGGTCCGTGGGAACTACCAACTT

         910        920
CAGACGGTTCACTCCAGAGTCGCTGGC
```

Appendix I:

LM-PCR primers: used for LM-PCR out (upstream) from the RACE products

CAGGTTGCCTCTACTGAAAGCCATGAGC –LMPCRScn10a-1
GCTCAGCATTCGCGCATGGCAGG –LMPCRScn10a-2
CTCCATCTGCCATCTGTTCTCCGTC –LMPCRScn10a-3
CTCCATCTGCCATCTGTTCTCCGTC –LMPCRScn10a-3a
CTCCGTCTAAAGGCCTGGGGTAGC –LMPCRScn10a

Sequence of combined two rounds of LM-PCR 5' from ends of RACE products:

```
         10        20        30        40        50        60
ATTCCAGTTGCTGAGTGGAGAGAGCACTGTAGGGTCATGGAAGGACAGTGGGGAGGTCTG

         70        80        90       100       110       120
TTAGAGGTCCTTGAAATTATATAGTGACCTCGCCATGATGGTGGTCTCAGAGATCGAGAG

        130       140       150       160       170       180
ATGATGTAATCAGGAGGACTCTAGGAATTCAAGTTAGAGGCCCCAGAAAGAGGGCTGTGG

        190       200       210       220       230       240
ACGAGGGACGGCTCTTGGATTACCTCTAGATGCTGGGCTTGTGAGTCCAGGCAAGCAGAG

        250       260       270       280       290       300
TGTTCTTGGAGAGGCTTCTCTGGGGGAGGATCATTCTGAGCAGGGCACAGGCACAGAAAT

        310       320       330       340       350       360
CATTAGTCCATCTGTAAACATGTCTGAGATGTTAGTGGAGTGTCCATGAAGGGAAATTCA

        370       380       390       400       410       420
GGCTTCTACCACATTAGTGTATATTTAAATCTGACACCAGGAGAGAGATTTATGATGGAG

        430       440       450       460       470       480
CTGACAGACTCCGGTGCCATGTCAGGTAGGTGACTGAAGCCCTGGGGAAGGAGAGGCGTA

        490       500       510       520       530       540
GGATGGAATCTTAAAACGATTCTCCAACTACTTCCAGGTGGCAGAGGAGGAGGCAGCCCA

        550       560       570       580       590       600
GGCCAGAGAAGCTCCTCTGAAAACAGAAGTCAAGAGGGTGGAGTGTGGTGCAAGGACCAT

        610       620       630       640       650       660
GCAGCTAATCCTGCGGAGCCCCTAGGATGAGAGCGCCAGAGAGGAGACACATGACACAGG

        670       680       690       700       710       720
GAGACCAGTAGAAACCTGTTAAGATTCCGGGTGTCTCAGGACTGCCTCTGGATGCACACT

        730       740       750       760       770       780
TCTTCCTTCTTGGGAAGTTACTTTTCTGTCACTGTGATGAAATACCTTAACCAAGGTGAC

        790       800       810       820       830       840
TCAAAGAAGAGAGGGTTTATCTGGGCTCACGGGTCCAGAGGTAGAGGAACACATGGAGAT

        850       860       870       880       890       900
CGTGGTGGGGAACCAGTGTAGCAGGCAAGCATGGTGGCTGGGGCTGAGGCTGAGAGCTTA

        910       920       930       940       950       960
TATCTTTGTCTGTATACAGAAAGCAGAGAGAGCCAACTGGGAATGACTTGTGGCTTTTGG
```

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      970       980       990      1000      1010      1020
AACCTGAAACCTGTCTTCGGTGACATGCTCCCTCCAGCGAAGGCAATGCCTCCTCAAACT

     1030      1040      1050      1060      1070      1080
CCCCAAAGGGCACCACAAACTAGGAACCAAGCACTCAGATGCCCGAGACTATGAGCGACA

     1090      1100      1110      1120      1130      1140
TCTCCTTCAGATCACCACACTTGGGTACACCCATTCTCCTGTCTCATCCAGTTTGCTCTT

     1150      1160      1170      1180      1190      1200
CTGGAAGGGTGGTGAGAGGGATGACAGCTAGTGACAAGTTGGAGAGACTTTAGAATAATT

     1210      1220      1230      1240      1250      1260
GCCATCACACAAAGCCTACCCTATCAGTTAGTGGCTGGCACGCTATCCCAACAGCTTGAG

     1270      1280      1290      1300      1310      1320
TCTGAACTTGCCAGAAATGGCCTCCGTCTCACCTCTCCCAGGCTCCCCAGCACCCACAGG

     1330      1340      1350      1360      1370      1380
TGCCCTCCCCCAAGACCTGACATCATCGGAGCACTGAAGAGATGCCTCCTCTGCCCCTTT

     1390      1400      1410      1420      1430      1440
CTCCCCTGGTCTGATTGCTACCAGGCAGCTGATCCACATGCCCTGCTCCAAGTTTGACCC

     1450      1460      1470      1480      1490      1500
CAGTCAGCAGGCTTCTCTGAAGAAGAGGGTCTGTTAGCATGACACACAGCATTTCCCATG

     1510      1520      1530      1540      1550      1560
CAACAGAACCTTGGAACCTAGGACAGAACTCAAGATATCAACGTGACACACACATGTACA

     1570      1580      1590      1600      1610      1620
TGTACCCTTACACACCTGAACGTGCATATACACACGTACACTTGTACACACACTAAAATA

     1630      1640      1650      1660      1670      1680
ACTGTGGTGCCAGGAGTGATCAGGGTACTGAGGTGACAGTCATTCACAAGGCTTGCCTTT

     1690      1700      1710      1720      1730      1740
CTCCACGTGACACACTTTGACCTTACCACCTGTTTCCACCTTGCTCTGGCATTTTAAAAA

     1750      1760      1770      1780      1790      1800
CATGACATTTTTAGTAAATTCTTTGAATTTTTTTGAGACAGGGTCTTACACTACAGCTCA

     1810      1820      1830      1840      1850      1860
AGCTGATCTTGGGTTTGCAGCAATCCTCTTCCCTCGACCACCCCCCCAACTAGGATGTGA

     1870      1880      1890      1900      1910      1920
GCTGCCATGCCCAGTTTGACTCTTTCCCAGATGTTTGTTTTTATTCTGTATGTATGAGTG

     1930      1940      1950      1960      1970      1980
TTCTCACTGTATGTATGTCTGTATATGCACCATGTAGAGCCCAAAAGCAGTTGCTGAATG

     1990      2000      2010      2020      2030      2040
CTCCGGAGCTTGGAGCTGTGGGTGGCTGTGAGCTGCCACGTGGGGTGCCAAGAATAGAAC

     2050      2060      2070      2080      2090      2100
ACAGGTCCTCTGCAAGAGCATCGAATGCTCTTAACCACTGAGCTATCCCTCCAGGTATTA

     2110      2120      2130      2140      2150      2160
AAACATAAAATAAGCTTGACTTTTTACTCTGACATAATTTAAAATTCACAAAAAGTTTTA

     2170      2180      2190      2200      2210      2220
AAAAAACGTAACAGCTTCCGTATACTCCCAATCCCATTTCCCCAGTTAGGATATTCTTTA

     2230      2240      2250      2260      2270      2280
ACCATAGTACATTGTCAAATGAGAAAACTAACATTCATACAACACGACTGATTTTGGTGA
```

```
        2290       2300       2310       2320       2330       2340
AAATCCTATTTGGAGTACACTACCTTTGACTGTGATTTCTTTTTGCACTATGGCCCAGGC

        2350       2360       2370       2380       2390       2400
TAGCCTGCAACTATTTATCATTATTTATCCCAGGGTGACTTGAACTTAGGGCAATTCTTC

        2410       2420       2430       2440       2450       2460
TACCTCAGCTCTGCCCCCACTCCCACCTCAACTCCAGTCCTGGGGTTACAGGAGCAAGCC

        2470       2480       2490       2500       2510       2520
ATCAAGTTCTATAACATTTAATACACAAGGACACTGGTTAAACTCAGAAGGACCTAAATT

        2530       2540       2550       2560       2570       2580
AGCATAAGACTATGGGGACCAGAGAAGTGAGAAGTGAGGACAGGGGAGGAGGGCAGGGGA

        2590       2600       2610       2620       2630       2640
GGGAAGATGGGAGGAATGATGGGAAGAGAATGAGAGAAGGCAGGGAGGGAGAGGAGAAGG

        2650       2660       2670       2680       2690       2700
CCAGTGAAGGGAGAATGGGAAGGGAGGGAGTTGAGAGAAGGCAGGATCGGGAGCCATAGA

        2710       2720       2730       2740       2750       2760
ATGTCTGTAGGAAACCATCAAAGGCATTTAATTTAATAAAGCAACCAGGATTGTACATAA

        2770       2780       2790       2800       2810       2820
TTCTACTGTGTACATACAAACACTCAAGTTTTGGGAGCAAGAATTTTAGCTTTCCTTCCC

        2830       2840       2850       2860       2870       2880
CTGCCCCTTTATGATTCAGTCTCTGCTAGAAAAAGTGGAGCCTTGCAGGGTGTGGTGGTG

        2890       2900       2910       2920       2930       2940
CACGCCTTTAATTCCAGCGTTTGGGAGGCAGAGGCAGGTGGATTTCTGTGAGTTCCCGGT

        2950       2960       2970       2980       2990       3000
CAACCAAATCTCCATAGTATGATCCTTCGTGGAATACCGGCCAACCAAACAAGCAAACAA

        3010       3020       3030       3040       3050       3060
ACAAACAAAAATCCCAAACAAACCCCACCCCCACCCAAATAGAGGGGATTATTGACTCAA

        3070       3080       3090       3100       3110       3120
AGAAGCCAATAATTTTGAGTTGGTTTGGGACATTTGAGTAAATGAAGCTGTAATGGGCAA

        3130       3140       3150       3160       3170       3180
GCATGGGCCCTCGACAGTTTCCTGCAGTATAGCATGGCTTCCTAAGGCTGCGTGGGTTGC

        3190       3200       3210       3220       3230       3240
ACTGTTACGGAGGGCTCAGCTCAGACAGGGGGTTCCCTGTGCAACCTCCTTTCTTATGGT

        3250       3260       3270       3280       3290       3300
CCCACAACCCCACAGATAGGGCACTTTCCCTACCCAGCTCCCTTCTCGGCTCTCACTGGG

        3310       3320       3330       3340       3350       3360
GTCGGAGAACATTTTTGTTTCAGCATTTCATCTGAAGCCACGGTTTCACATCATCAAGTC

        3370       3380       3390       3400       3410       3420
TGCAAAAAACCGTTCACAAACCACACCAGAACTTCTCGGTAAAGAACTCCTAAGACCAAA

        3430       3440       3450       3460       3470       3480
GAGGGAGACTGGGTAGATTGTTTTTAATTTGTTTCTTTTTGTCAAAGGGGGACAAAACAC

        3490       3500       3510       3520       3530       3540
GCTTTGGTGAGTGCGAGTGTTTATTCTGGGACACAAACCCAGAGTCTGGAAGGGAGCATT

        3550       3560       3570       3580       3590       3600
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CAACGGGTGCTGCTCTGCCACGCAGGGGCAGCGGTGGGACTCAGCCCATCCTGCTAAGGA

```
       3610      3620      3630      3640      3650      3660
CGGGCAGCCTGAGCCAGGCTTGGGAGTCTGTCATGGCTGCCAGACGAATCATTATCTAAT

       3670      3680      3690      3700      3710      3720
TGCAGCCTTTTCTCTTCCTTAGGTTTCAGCAGGTCCCGAGAGAGCATTTAAAATCGCATT

       3730      3740      3750      3760      3770      3780
TACTACTTTACCATCTAATCACACATAAGCCTCTCCCTATACCCTCCACCCTCCTTCCAT

       3790      3800      3810      3820      3830      3840
TCAGAGTGTACTTTCTGGAGCCCATCCAGCAAGCAGGGTGGAACTCATGACGGGAAATGG

       3850      3860      3870      3880      3890      3900
GAACGGCGCCCACGAAGGCGTGATTCCTTGTAGATCCTTGAGTGATGGACGGGTGAGGTT

       3910      3920      3930      3940      3950      3960
TCCGTCAGGCAAGCCCAGCCACCTTCGTGGAGGAGCCCCGGACAAGTGTAAGTTTCGCAG

       3970      3980      3990      4000      4010      4020
AGCTGGGGTCTCCAGCTTACTTCTGCTAATGCTACCCCAGGCCTTTAGACGGAGAACAGA

       4030
TGGCAGATGGAG
```

Appendix J:

Primers for genomic screening of mouse library:

CCTGTGTGTGCTGTAAAAAGGATC - EX1-3' OUT
TGAGAAGATGGAGTTCCCCTTTGG – EX1-5' IN

Amplified fragment for PCR screening of mouse genomic library:

```
          10        20        30        40        50        60
TGAGAAGATGGAGTTCCCCTTTGGGTCCGTGGGAACTACCAACTTCAGACGGTTCACTCC

          70        80        90       100       110       120
AGAGTCGCTGGCAGAGATCGAGAAGCAGATCGCTGCCCACCGCGCCGCCAAGAAGGGCAG

         130       140       150       160       170       180
AACTAAGCAAAGAGGACAGAAGGACAAGAGTGAGAAGCCCAGGCCTCAGTTGGACTTGAA

         190       200       210       220       230       240
GGCCTGTAACCAGCTGCCCAGGTTCTATGGCGAGCTCCCAGCAGAGCTGGTCGGGGAGCC

         250       260       270
CCTGGAGGACCTGGATCCTTTTTACAGCACACACAGG
```

Souslova VA, Fox M, Wood JN, and Akopian AN "Cloning and Characterization of a Mouse Sensory Neuron Tetrodotoxin-Resistant Voltage-Gated Sodium Channel Gene, Scn10a" *Genomics* **41**, 201-209 (1997).